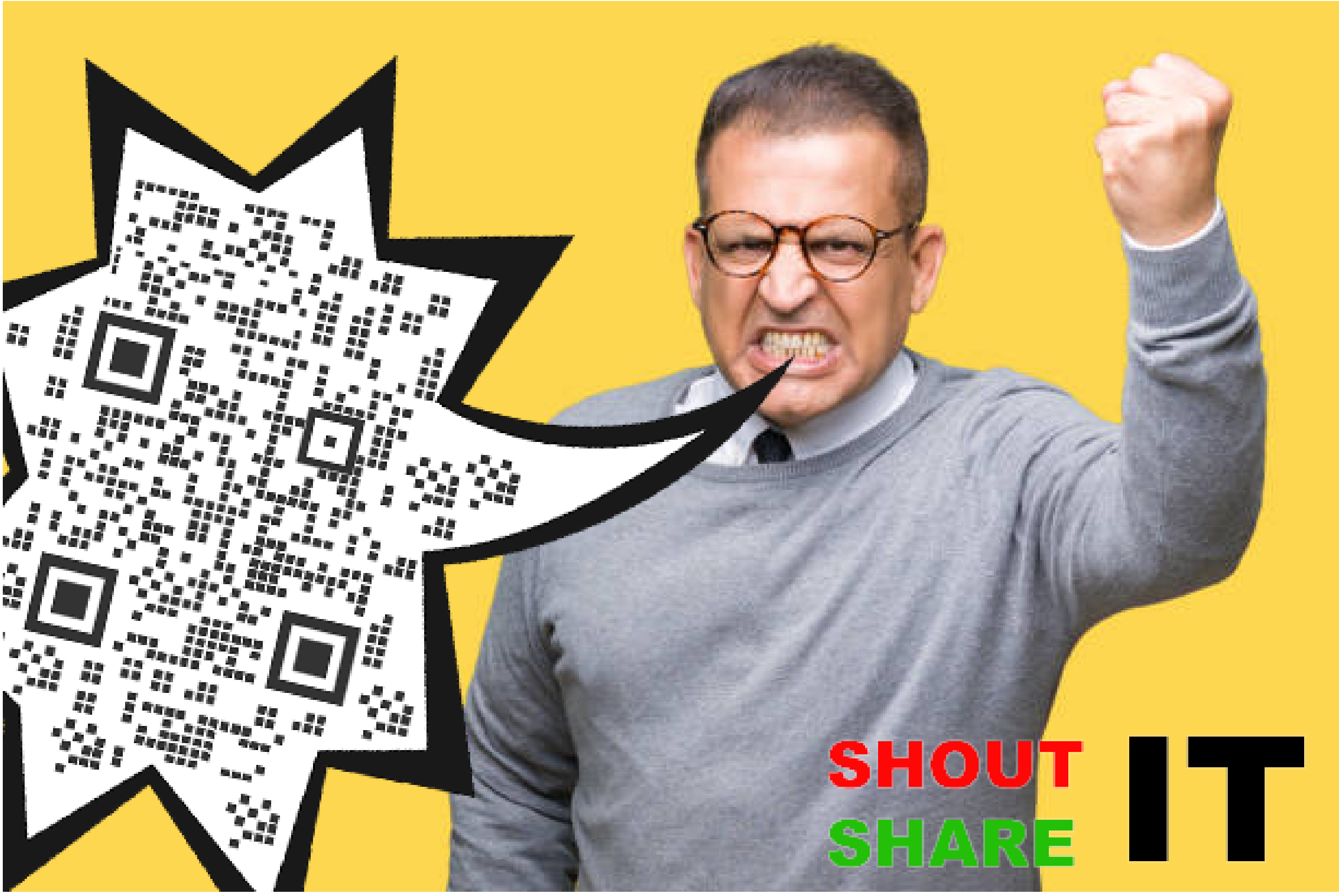
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AT

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Bulletin of the Museum of Comparative Zoölogy
AT HARVARD COLLEGE.
VOL. XLV. No. 1.

THE ANATOMY OF HEMIURUS CRENATUS (RUD.) LÜHE, AN APPENDICULATE TREMATODE

BY CLARENCE H. LANDER.

WITH FOUR PLATES.

CAMBRIDGE, MASS., U.S.A.:
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JANUARY, 1904.

No. 1. — CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, UNDER THE DIRECTION OF E. L. MARK, No. 148.

The Anatomy of Hemiurus crenatus (Rud.) Lühe, an Appendiculate Trematode.

By Clarence H. Lander.

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Introduction.

This species of appendiculate distome was first described by Rudolphi (**'02**, pp. 76–78) as Fasciola crenata, and later (**'09**, pp. 404, 405, tab. 5, fig 1.) as Distoma crenatum. It has since been included under Distomum (or Apoblema) appendiculatum by Rudolphi (**'19**), Monticelli (**'91**), Mühling (**'98**), and also under Distomum ventricosum by Wagener (**'60**). It is described as Distomum ocreatum by Olsson (**'67**), and Linton (**: 00**), as Apoblema ocreatum by Juel (**'89**), and as Hemiurus ocreatus by Looss (**'99**). The name Hemiurus crenatus was first applied to it by Lühe (**: 01**), who gives its synonomy and a brief characterization of the species. The genus Hemiurus was established by Looss (**'99**), who includes under it all appendiculate distomes except those lacking an appendix or having an extremely small one. Lühe proposes to raise the genus to the rank of a family, under which he would include twelve genera. He describes three species belonging to Hemiurus s.str. and mentions two others which are perhaps also to be included in the genus. It is very doubtful if the specimen shown in Olsson's Figure 98 is Hemiurus crenatus, since the ventral sucker is represented as twice as large as the oral sucker, while in all the specimens I have examined the suckers are very nearly equal in size. In Linton's Figure 19 (Plate 35) the pars prostatica is represented as being four or five times as long as it is in any specimens I have studied, and I think it probable that some of the specimens referred by him to Distomum ocreatum do not belong to this species.

My studies have been made on specimens taken from the stomach of both Anguilla chrysypa Raf.[1] and Osmerus mordax Mitchill during the months of October and November. The parasite is by no means common, as I have frequently examined fifty or more stomachs without finding a single specimen. When present, the number found in a single host varied from one to fifteen or twenty. In December I obtained very few specimens, and after that did not succeed in finding any, although I repeatedly examined stomachs of the infected species until about the middle of May.

Hot corrosive sublimate was found to give the best results as a fixing agent for specimens to be sectioned, though it was not at all satisfactory as a fixative for specimens to be mounted entire. For the latter purpose Kleinenberg's picro-sulphuric mixture gave the best results. Alum carmine and Brazilin proved most satisfactory for staining sections and Delafield's haematoxylin for entire preparations. The work has been carried on under the direction of Professor Mark, to whom I am indebted for many suggestions and much valuable assistance.

External Characters.

Upon measuring seven preserved specimens with the appendix extended (compare Fig. 1), it was found that the average length was 2.6 mm., the extremes being 1.71 mm. and 3.219 mm. The average width was 0.323 mm., the extremes being 0.222 mm. and 0.444 mm. The average length of the trunk was 1.982 mm. and that of the appendix 0.799 mm., the appendix being therefore a little more than three-eighths as long as the trunk. Lühe (**:01**) states that the appendix reaches about three-fourths the length of the trunk.

The ventral and oral suckers are very nearly equal in size. From measurements made on five specimens, it was found that the average diameter of the oral sucker was 0.224 mm., while that of the ventral sucker was 0.2646 mm., though in some cases the oral sucker is equal in size to the ventral or even a little larger. The oral sucker is at the anterior end and faces antero-ventrally. From measurements on the five specimens, it was found that the distance of the centre of the ventral sucker from the anterior end of the body was from one-fourth to one-seventh of the entire length of the worm. The genital pore lies ventral to the posterior end of the pharynx, about midway between the two suckers, or a little nearer the oral sucker. Slightly in front of the ventral sucker is a deep, transverse, slit-like depression, which may be designated as the preacetabular fossa. The width of the fossa is slightly less than that of the ventral sucker. A characteristic feature of this, as well as of several other species of the Hemiuridae, is the ringed appearance of the trunk. The length of these rings varies according to the state of contraction of the body, those near the posterior end of the trunk being, however, several times as long as those near the anterior end. In one specimen 142 of these rings were counted. The appendix is not ringed, but usually presents one or two slight constrictions.

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[1] Rafinesque (**'17**) spelled this name *chrisypa*.

Body Wall.

The wall of the body exhibits the usual structures: viz. a cuticula, or external structureless covering, a layer of circular muscle fibres, a layer of longitudinal muscle fibres, and a layer of diagonal muscle fibres.

An inner and an outer layer of the cuticula, commonly described, are not distinguishable in this species. The rings noticed on the surface of the body are due to variation in the thickness of the cuticula. The thickness at the anterior end of a ring is about $3.6\,\mu$, whereas at the posterior end of the ring it is from two to four times as great (Plate 1, Fig. 5), so that when seen in profile a notch marks the posterior end of each ring.

The outer (circular) layer of muscles of the body wall consists of very small fibres (about $0.5\,\mu$ in diameter) so closely set that in transverse sections they appear to form a continuous sheet and in longitudinal sections a row of minute dots. They are best seen in tangential sections (Plate 3, Fig. 34).

The longitudinal fibres are much larger, measuring $2\,\mu$ to $4\,\mu$ in diameter. They are circular or oval in cross section, and in good preparations show a clear central area, surrounded by a more deeply staining highly refractive portion.

Trematode muscle fibres have been described by several authors (Juel, '89; Pratt, '98) as having a structure similar to this. None of the muscle fibres of the body wall show any traces of nuclei.

The layer of diagonal muscle fibres is quite well developed. In transverse sections the diagonal fibres are not easily distinguished, but their size and arrangement are well shown in tangential sections (Plate 3, Fig. 34). They are slightly larger than the longitudinal fibres. There are two series of fibres, which cross each other at nearly right angles. The distance between the fibres is such that the meshes are from two to four times as wide as the fibres. Fibres of all three layers are occasionally seen to branch.

In the appendix the body muscles are less strongly developed than in the trunk, and the oblique muscle fibres are lacking. I find, as stated by Juel ('89), that the relative positions of the circular and longitudinal muscle fibres are the reverse of those found in the trunk, the longitudinal fibres of the appendix being next to the cuticula (Plate 2, Fig. 14). Juel calls attention to the agreement in position between the muscles of the appendix and those of the excretory vessel, and suggests that the muscles of the two have developed in connection with each other. I think his suggestion quite pertinent and believe that the inversion of the muscle layers of the appendix is significant as to the method in which the appendix originated. Pratt ('98) believes that the appendix is the whole, or a part, of the excretory vesicle in an evaginated state. In young specimens of Apoblema [Hemiurus] appendiculatum which are as yet without an appendix he finds at the posterior end of the body what he calls an appendicular vesicle, a sack lined with a high columnar epithelium. At its posterior end, this sack opens to the exterior by a pore guarded by a sphincter, while into its anterior end the excretory vesicle opens. Later this appendicular vesicle becomes evaginated to form the appendix and its epithelium is sooner or later shed.

In Hemiurus crenatus and other appendiculate distomes, so far as they have been described in this particular, the excretory vesicle is provided with longitudinal muscle fibres which lie next to its structureless lining, and surrounding these are circular muscle fibres. Upon evagination of the excretory vesicle the muscles of its wall would appear as body muscles, the outer layer consisting of longitudinal fibres and the inner layer of circular fibres, which is precisely the condition found in the appendix, but the reverse of that found in the rest of the body. It seems to me, therefore, that Pratt's view as to the origin of the appendix furnishes a satisfactory explanation of the difference between the arrangement of the muscle layers of the appendix and those of the trunk, — a condition for which it seems difficult to offer any other explanation.

The muscle fibres of the suckers are well developed, and both the radial and peripheral fibres show a clear central area, while the peripheral portion of the fibre shows a fibrillar structure. Side views of fibres show clearly a longitudinal striation, and in cross section the peripheral portion of each fibre has the appearance of minute dots.

The longitudinal muscle fibres of Ogmogaster have been described by Jägerskiöld ('91) as consisting of fibrillæ, while he describes the circular muscle fibres as showing a clear central area and a peripheral, highly refractive portion, the same condition as that described above for the longitudinal muscle fibres of Hemiurus crenatus. Walter ('93) describes both the dorso-ventral muscle fibres and the muscle fibres of the body wall as having a fibrillar structure. According to Stafford's ('96) account the dorso-ventral muscles of Aspidogaster have a fibrillar structure, and also show in cross section a deeply staining peripheral portion surrounding a less deeply staining core.

There is a special development of muscle fibres in connection with the preacetabular fossa. The structure consists of a mass of radially arranged fibres between which is found a finely granular substance (Plate 3, Fig. 28). The greater portion of the structure lies anterior to the fossa. There is no internal limiting membrane, as in the case of the suckers, and many of the fibres pass out some distance into the parenchyma.

Parenchyma.

The parenchyma presents three principal modifications, a peripheral granular layer, a vesicular parenchyma, occupying most of the space between the organs, and a cellular sheath found around the intestinal coeca in the posterior portion of the body (Plate 2, Fig. 25). The peripheral granular portion, or sub-cuticula, forms quite a distinct layer next to the body muscles, and it fills in the spaces between the muscle fibres. It appears to be rather irregularly but finely granular, and is from one to three times as thick as the cuticula (Fig. 25, *pa'ench. gran.*). The layer as a whole shows no evidence of cellular structure, but imbedded in it are numerous groups of nuclei, each group being surrounded by a circumscribed mass of protoplasm (Plate 2, Fig. 25; Plate 3, Figs. 29–31). In each of these clusters can be counted from one to ten nuclei, the larger groups being the more common. In the majority of cases each group appears to constitute a syncytium, but in many groups the protoplasm is distinctly divided into cells (Fig. 31). Corresponding to each group of nuclei there occurs a thickening of the peripheral granular layer, which protrudes into the vesicular parenchyma. Each nucleus shows a large number of small deeply staining granules, which are especially abundant in the periphery, and usually one much larger chromatic mass, the diameter of which may be one-fourth as great as that of the nucleus. The protoplasm surrounding the nuclei is sharply marked off from the granular parenchyma in which it is imbedded, and it appears in most cases to be homogeneous, but in some groups it has a finely granular appearance. These groups of nuclei are quite uniformly distributed in the peripheral granular layer over all parts of the body, and similar groups are also found between the muscle fibres of the pharynx and suckers and occasionally in the vesicular parenchyma, especially in the anterior portion of the body.

There are also found in the granular layer of parenchyma somewhat larger oval, pyriform, or spindled-shaped cells (Plate 1, Figs. 4, 7–13). These cells are much less numerous than the subcuticular cells, as a rule lie deeper than they do, and never occur in large groups, though sometimes two may lie close together (Fig. 11). The protoplasm stains deeply with carmine and shows large, often flake-like granules. The nuclei are large and clear save for the single nucleolus, which is generally oval in form and unusually large, its length being often nearly as great as the diameter of the nucleus. The long spindle-shaped cells (Fig. 4) are undoubtedly destined to become parenchyme muscle fibres, and the shorter cells, which are usually the smaller ones, may be very well regarded as earlier stages in the formation of such fibres. The long axis of these cells is usually parallel to the surface of the body, as is often the case with the parenchyme muscle fibres.

A third kind of cell to be found in the granular layer is the giant cell, the so-called "grossen Zellen" of German authors. The cells in Hemiurus which I take to be giant cells resemble somewhat the granular cells just described. Their cytoplasm stains but slightly, and is usually continued into several processes (Plate 4, Figs. 39–41). There is a great variation in the amount of cytoplasm around the nucleus, in some cells the cytoplasmic portion of the cell being very large, while in others it forms such a thin sheath around the nucleus as to be scarcely apparent. According to the recent researches of Bettendorf ('97) these cells are to be regarded as myoblasts, lying, as a rule, at some distance from their differentiated contractile portions, with which they are connected by numerous, delicate, branching processes. If we regard the giant cells as myoblasts of the muscles of the body wall and the granular cells as in process of development into parenchyme muscle fibre, i. e. as myoblasts of parenchyme muscle, it is not at all surprising to find a close resemblance between them. It is to be noted, however, that the method of formation of the muscle fibres is quite different in the two cases. In one the contractile elements become differentiated in such a manner that the body of the cell lies external to them; there are usually several contractile elements or fibres formed by each myoblast, and each fibre remains connected to it by a protoplasmic process. In the other case the contractile substance is formed in the entire periphery of the spindle-shaped myoblast, thus enclosing the body of the cell. Each cell in this case results in the formation of a single fibre.

Poirier ('85) describes and figures groups of cells found in Distomum insigne and D. megnini, similar to the subcuticular cells of Hemiurus crenatus, and states that they are probably of a glandular nature, though he was unable to find any traces of ducts.

Prenant ('86) describes a peripheral granular layer of parenchyma in Distomum (Lecithorchium) rufoviride which is very similar to that present in Hemiurus crenatus. He regards it as a protoplasmic myogenic layer, which, though provided with groups of nuclei, is not in a cellular condition, and he feels compelled to conclude that muscle fibres have been formed by a differentiation *en bloc* of the myogenic protoplasm. The conditions found in Hemiurus crenatus differ from those found by Prenant, in that each nucleus, or group of nuclei, is surrounded by a definitely limited mass of protoplasm, which is distinct from the granular layer, so that these nuclei cannot in this case be regarded as the nuclei of the granular parenchyma. I am unable to offer any satisfactory explanation of the source of this layer, but it certainly cannot be regarded as the cytoplasm of the nuclei of the cells imbedded in it. According to other investigators, as mentioned above, the muscles of the body wall are developed from cells which remain as the giant cells.

Juel ('89) in his description of Apoblema excisum, A. appendiculatum, and A. rufoviride, does not mention anything which would correspond to the peripheral granular layer of Hemiurus crenatus, but finds in each species underneath the entire body musculature smaller or larger groups of finely granular cells, similar in appearance to the subcuticular cells of Hemiurus crenatus. He believes the function of these cells is to furnish nourishment for the cuticular and the muscle layers.

Stafford ('96) describes somewhat similar cells, which he terms peripheral parenchyme cells. They appear exactly like the cells of the embryo, the protoplasm being hyaline. He also finds cells that show a transition from these to the ordinary parenchyma; he thinks it possible that some of the peripheral cells metamorphose into glands.

Pratt ('98) finds in young Apoblema appendiculatum immediately beneath the muscles of the body wall a more or less scattered layer of cells, which are oval or pyriform in shape. He terms them submuscular cells, and is of the opinion that they are unicellular glands which furnish a substance serving to render the cuticula immune from the disintegrating action of the body fluids of the host. He states that these cells are lacking in the appendix; but in Hemiurus crenatus the subcuticular cells are as abundant in the appendix as in any other region of the body.

In Hemiurus crenatus the subcuticular cells give no indication whatever of being glandular. I find no traces of ducts, their cytoplasm is homogeneous or very finely granular, and they commonly form syncytia rather than distinct cells. I have, however, been unable to find any cells which I could identify as the Hautdrüsen, or dermal glands, commonly described in trematodes. I believe the subcuticular cells of this species are primitive, undifferentiated cells, a very large number of which persist throughout the life of the worm. It seems probable that all the cells or nuclei of each group arise from the division of a single cell or its nucleus; and although none were observed in process of division, there is nothing in their appearance to suggest that they have lost the power of division. Thus the subcuticular cells, as Looss ('93) suggests, correspond anatomically and physiologically to the cambium of plants. As will be suggested later, I think that they probably give rise to myoblasts, but I have found no indications either of their degeneration, of their development into vesicular parenchyma, or of their conversion into gland cells.

The oval and spindle-shaped granular cells of this layer are probably cells in process of development into parenchyme muscle fibres. The development of these muscle fibres from oval cells lying in the parenchyma has been observed by Poirier ('85) in young specimens of Distomum clavatum and D. verrucosum. He finds all stages from the oval cells to the almost entirely formed muscle fibre. The nucleus and cytoplasmic portion of the cell are surrounded by the contractile portion, which he describes as being formed by a thickening of the wall of the cell. According to Poirier, both granular protoplasm and nucleus entirely disappear, though there remains for a long time, and even in adult animals, a central core differing noticeably in appearance from the peripheral portion.

The parenchyme muscle fibres described by Macé ('82) and those described by Stafford ('96), in which the body of the muscle cell lies on one side of the contractile fibre, suggests another method of development of parenchyme muscle fibres, which resembles the supposed process of development of the muscles of the body wall, in that the body of the cell remains external to its contractile portion.

As to the source of these myoblasts, I am inclined to believe that they are derived from the subcuticular cells, which, as stated above, I believe to be undifferentiated embryonic cells persisting in the adult. It seems probable that, up to a late stage in the life of the worm, they continue to give rise to cells which become differentiated into muscular elements and possibly into other tissues.

A striking feature of the parenchyma in this species is the large cells which form a sheath around the two intestinal coeca from the region of the vitellaria to their posterior ends (Plate 2, Fig. 25, *pa'ench. cl.*). The protoplasm of these cells is distinctly granular, and each shows a clear spherical or oval nucleus provided with a single chromatic granule. They are usually arranged in a single layer around each of the crura; occasional cells occur in the parenchyma anterior to the point where the sheath ceases. I have been unable to find in any descriptions of the parenchyma of trematodes an equally extensive tissue which has retained so perfectly its cellular condition. The parenchyma of the posterior portion of the body, however, is often spoken of as being least modified.

The remaining space between the various organs and the peripheral granular layer is occupied by vesicular parenchyma (Plate 2, Fig. 25, *pa'ench.*), which usually exhibits a quite regular network, the meshes of which undoubtedly correspond to the cells from which the tissue has been derived; the walls forming the network are obviously the walls of those cells. I have found no nuclei in the vesicular parenchyma: the bodies which in transverse sections appear at first sight to be such, prove on closer examination to be the cut ends of parenchyme muscle fibres (Fig. 25, *mu. rtr.*). In the posterior portion of the body there are scarcely any traces of granular cytoplasm, the meshes in life being filled probably with a watery fluid. In the anterior portion of the body considerable granular substance may be found in the meshes of the parenchyma, and the vesicular parenchyma often passes more or less gradually into the peripheral granular layer. The meshes are of various sizes, some of them being as large as the cells ensheathing the crura, but the most of them smaller and, as a rule, more or less compressed.

Anterior to the vitellaria the meshwork is not so regular as in the posterior portion of the body, and in front of the ventral sucker occasional cells similar to the subcuticular cells occur scattered throughout the vesicular parenchyma. The longitudinal parenchyme muscle fibres which form the retractors of the appendix are a characteristic feature in the posterior portion of the body. They pass through the walls of the meshes of the vesicular parenchyma.

Many of the dorso-ventral parenchyme muscle fibres are provided with a centrally situated nucleus surrounded with granular cytoplasm, while the peripheral portion of the fibre has a homogeneous appearance (Plate 1, Fig. 3). In some cases, however, the entire fibre shows a coarsely granular condition (Fig. 2). In cross section the large fibres show a peripheral contractile portion and a central colorless core (Plate 1, Fig. 6), as described in the case of the longitudinal muscle fibres of the body wall. Many of the dorso-ventral muscle fibres lie along the inner margin of the peripheral granular parenchyma (Plate 2, Fig. 25, *mu'.*); and they frequently occur in pairs, one on either side of the body.

Very conspicuous in the posterior portion of the trunk and in the appendix are the long longitudinal parenchyme muscle fibres. They constitute the retractors of the appendix, either passing between the parenchyme cells or through their walls (Plate 2, Fig. 25, *mu. rtr.*). In cross section they give at first glance the impression of nuclei lying in the network. I could find no nuclei in these fibres.

Macé ('82) describes the parenchyme muscle fibres of Distoma [Fasciola] hepatica as consisting of two parts, — a very refractive, hyaline, cylindrical fibre of considerable length, and a small protoplasmic mass enclosing a nucleus and lying on the side of the fibre near its middle. He regards the cellular body as the remains of the primitive cell, which decreases in size as the fibre grows and very probably disappears altogether.

Stafford ('96) finds that the myoblasts of the dorso-ventral muscle fibres of Aspidogaster cling to the sides of the fibres, there being a single myoblast to each fibre. The surface of the fibres shows a longitudinal fibrillar structure, and in section the fibres appear as small deeply stained rings filled with a less deeply stained substance. He says nothing in regard to the possible disappearance of the myoblasts and evidently regards the conditions described as permanent.

Poirier ('85) finds parenchyme muscle fibres with a centrally situated nucleus, but believes that the nucleus disappears.

Kerbert ('81) also states that the spindle-shaped parenchyme muscle fibres of Distomum westermanni show a distinct oval nucleus, which is apparently centrally situated, though he does not so state.

Whether or not the nuclei observed in the dorso-ventral muscle fibres of Hemiurus crenatus ultimately disappear, I am not prepared to say. They are certainly present in many apparently functional fibres of mature worms.

Digestive System.

There is no prepharynx, the pharynx opening directly into the dorso-posterior part of the oral sucker. The pharynx is relatively quite large, varying from 0.108 mm. to 0.126 mm. in diameter and from 0.117 mm. to 0.144 mm. in length.

There is a short œsophagus, in spite of frequent statements to the contrary. In preserved specimens it turns dorsally from the posterior end of the pharynx, then bends anteriorly and extends to about the middle of the dorsal surface of the pharynx, where it bifurcates to join the intestinal coeca. It is provided with a thick lining resembling cuticula, which also extends a short distance into each of the coeca, where it ceases abruptly at the beginning of the intestinal epithelium (Plate 3, Fig. 35, *cta'*.).

The intestinal coeca (Fig. 1) pass from the end of the œsophagus a short distance toward either side of the body, then turning backwards extend to near the posterior end of the appendix. There is a slight constriction between the transverse and longitudinal portion of each coecum (Fig. 35). The condition of the intestinal epithelium is almost identical with that described by Juel ('89) for the species of Apoblema which he studied. In the anterior transverse portion of the coeca it has the appearance of long, closely packed threads with a few small conical granular masses next to the basement membrane (Plate 3, Fig. 35). As Juel suggests, I believe these are the basal portions of the epithelial cells whose inner portions have become modified into the fine protoplasmic threads. Looss ('94, p. 142) describes a similar condition of the intestinal epithelium of several distomes and speaks as though the entire epithelium was so modified. Looss shows nuclei in his figure; Juel does not. I do not find any nuclei in this part of the epithelium, but deeply staining bodies are present, the significance of which will presently be discussed. The epithelium of the greater part of the coeca consists of slender, nearly cylindrical cells, usually becoming smaller toward their free ends (Plate 2, Fig. 25; Plate 3, Fig. 36), but, as Juel states, in some places the cells appear to have fused together into a common mass. According to Juel many of the cells contain each a small nucleus, but many appear to be without nuclei. I have been unable to distinguish any nuclei in the epithelial cells of Hemiurus crenatus, but I have found in every specimen sectioned, as well as in several preparations examined without sectioning, numerous, deeply staining, spherical, oval, or club-shaped, nucleus-like bodies (Plate 3, Fig. 36), which in most cases are clearly seen to be situated between the cells. Each is usually in contact with the basement membrane or provided with a stalk, or style, which connects it with that structure, and the longer bodies often project as far into the lumen of the coecum as do the surrounding cells, or even farther. Each of the bodies is provided with a definite limiting wall, and contains one or more irregular darkly colored corpuscles. It seems difficult to interpret these bodies as cells; on the whole they have rather the appearance of nuclei. In all cases they are very uniformly distributed throughout the length of the coeca, which suggests that they might possibly be the nuclei of the epithelial cells. This does not seem probable, since in many cases they are clearly seen to lie between the cells, though of course in places where the cell boundaries are apparently obliterated it is impossible to determine their position in this respect. Moreover, the size of the bodies is such as to render it probable that they are not the nuclei of these cells, for they are often larger than the cells themselves. It has occurred to me that they might be nuclei of the cells from the host which had been taken by the worm into the coeca, where they are undergoing a process of digestion. In the slimy matter which often adheres to the body of the worm there are frequently found cells — probably mucous cells from the host — and immense numbers of nuclei without any surrounding cytoplasm. Many of these nuclei are very similar to those of the accompanying cells; others are more or less modified, but I believe that all are derived from such cells and that their present condition is due to degeneration. The modified nuclei are very similar in size, form, and appearance to the bodies found among the cells lining the crura, and I think it quite possible that the latter are nuclei of this kind which have been taken in by the worm. However, it seems almost incredible that every worm should have such a supply of them and that they should be distributed throughout the digestive tract with such great uniformity. Besides, if they are simply particles of food in process of digestion, it is difficult to understand why they should be found in such constant and intimate relation to the basement membrane. Furthermore, I have never noticed any indication of such a process of disintegration as might be expected if they were really in process of digestion. A further possibility is that the bodies are parasites, but in that case they would have at least the morphological value of cells; but, as stated before, it seems impossible to regard them as such. The same objection precludes the possibility of considering these bodies gland cells or otherwise modified epithelial cells of the coeca.

Immediately outside the basement membrane of the coeca I find a layer of circular muscle fibres (Plate 3, Figs. 35, 36), and outside these a layer of longitudinal muscle fibres (Plate 2, Fig. 25). The fibres are thread-like and lie some distance apart in both layers.

Excretory System.

The excretory system consists of a posterior sack-like reservoir, or vesicle (*vsl. exc.*, Plate 1, Fig. 1; Plate 2, Fig. 14,), into which opens a main ventral trunk that bifurcates above the ventral sucker into a pair of vessels which reunite dorsal to the pharynx, thus forming a loop that encircles the anterior portion of the digestive system. Aside from the small vessels the excretory system has in general the form of the frame of a tennis racket.

The excretory vesicle is from one-fifth to one-fourth as long as the appendix and has a diameter about twice as great as that of the excretory trunk which leads into it. The wall of the vesicle (Plate 2, Fig. 14, *vsl. exc.*) consists of three layers: internally a structureless membrane, immediately surrounding this a layer of delicate longitudinal muscle fibres, and outside the latter a layer of very large band-like circular muscle fibres. At its posterior end the vesicle opens to the exterior by a terminal pore, which, so far as I can make out, is not provided with a sphincter muscle, nor does there appear to be any vestibule present. At its anterior end the vesicle communicates with the excretory trunk by a narrow opening that is provided with a thick spool-shaped wall, which may possibly act as a valve (Plate 2, Fig. 14, *vlv. exc.*). This valve-like structure consists of a homogeneous substance resembling cuticula, and the short lumen through it, connecting the excretory vesicle with the excretory trunk, is very narrow. The wall of the valve is so thick as to render any great enlargement of the lumen highly improbable; moreover it appears to be distinct from the thin structureless membrane that lines vesicle and trunk, and passes continuously from one to the other. As there are no circular muscles surrounding the valve, I do not see how the lumen through it can be closed; but it seems probable that by a forcible contraction of the excretory vesicle, brought about by means of its strong circular muscle fibres, the greater part of the contents would be forced to the exterior through the much larger terminal pore, by which very little resistance would be offered.

The median excretory trunk extends from the excretory vesicle to a point dorsal to the ventral sucker, where it bifurcates; the branches turn toward the sides of the body and run forward nearly to its anterior end. Here each branch turns toward the median line and joins its mate dorsal to the anterior portion of the pharynx. The excretory trunk and the two branches which form this loop correspond, I believe, to the collecting tubes or large excretory vessels of other trematodes. In sections the wall of the vessel (Plate 2, Fig. 25, *va. exc.*) appears to be a structureless membrane, but occasional large oval nuclei are seen projecting from the wall into the lumen of the vessel. In some sections I have been able to make out very delicate circular and longitudinal fibres, and from the peristaltic contractions of the vessel in living worms it seems probable that it is provided throughout its length with such fibres.

I find a few small vessels arising from the lateral portions of the loop-like vessel, but these could be followed only a short distance through the parenchyma. Juel ('89) also found a number of small vessels, and Pratt ('98) describes a pair of delicate branching longitudinal vessels in living animals, though he was unable to make out their connection with the larger vessels.

There is considerable confusion in the interpretation of the parts of the excretory system of appendiculate distomes. Olsson ('67) appears to be the only one who has seen the excretory vessel, and he simply mentions the presence of a vesicula caudalis in the tip of the tail of Distomum [Hemiurus] appendiculatum. Juel ('89) states that he was unable to find any excretory vesicle in the species that he studied and he suggests that the vessel may itself occasionally become temporarily enlarged, giving the appearance of a vesicle. He therefore believes an excretory vesicle is wanting in appendiculate distomes and regards the entire racket-shaped structure as excretory vessels or collecting tubes.

Looss ('96, p. 123), in describing the excretory system of Apoblema mollissimum, states his belief that the racket-shaped portion of the excretory system represents the excretory vesicle. Pratt ('98) speaks of the two branches which form the loop in Apoblema appendiculatum as if they were a part of the vesicle proper (*i. e.* the excretory trunk), though he believes it would be more in harmony with the custom of authors to limit the application of the term vesicle to the unpaired portion of the system and to call the branches and their anterior uniting ends the collecting tubules. He adds that the entire structure forms a single organ, and no part differs from any other part in structure or function. Monticelli ('91) refers to the racket-shaped part as the trunk of the excretory system, and Linton (:00) speaks of it as the excretory vessel.

Under these conditions I fail to see the harmony that exists in the application of terms to this type of excretory system, and I do not find a single author who limits the term vesicle to the unpaired portion of either the Y or racket-shaped excretory systems and designates the branches as the collecting tubules. Braun (see Pagenstecher, A., und Braun, M., '87–93) states that there are cases in which the branches of the excretory vesicle pass gradually into the collecting tubes, so that it is impossible to say just where one stops and the other begins. He suggests that careful histological study may enable one to distinguish the two parts, but for the present he believes that the best way to distinguish them is by means of their contents. The vesicle contains larger or smaller refractive granules, while the collecting tubes never contain these, but are filled with a transparent or yellowish liquid which may contain fine granules. Accordingly he considers the entire racket-shaped portion of the excretory system to be a tube-like excretory vesicle.

As noted above, Looss ('96) and Pratt ('98) agree with Braun in this respect, but, as already described, the posterior part of the unpaired portion of the excretory system of Hemiurus crenatus is differentiated from the rest of the unpaired portion, and I believe this morphological differentiation fully warrants me in considering it as the excretory vesicle. The remainder of the racket-shaped portion I regard as the collecting tubes, the excretory trunk or unpaired portion having probably arisen by a fusion of the posterior portions of the two tubes.

Both Pratt and Juel found delicate muscle fibres on the wall of the excretory vessel. Pratt could not make out any definite arrangement, but Juel mentions two layers.

Pratt, in comparing the appendicular vesicle, i. e. the unevaginated appendix, with an excretory vesicle, speaks of its unusually thick walls as though that feature might be regarded as an objection to considering it an excretory vesicle. I have seen in young distomes, of a species found encysted in the crayfish, a high columnar epithelium, the cells of which are relatively much higher than those of the epithelium of the appendicular vesicle as described by Pratt. The epithelium in the distome referred to is also shed, and the shedding takes place at the time the parasite enters its final host or shortly before. I am inclined to believe that a similar process takes place in very many trematodes, since the wall of the excretory vesicle is often described as a structureless membrane without any traces of cells or nuclei. It is also interesting to note that in the case cited the lining of the excretory vesicle was shed at about the same time in the life of the parasite as was the epithelium of the appendix or of the appendicular vesicle as observed by Pratt.

Male Sexual Organs.

The *testes* (Fig. 1, *te.*) are spheroidal, and lie anterior to the middle of the body and a short distance posterior to the ventral sucker. They are obliquely placed, the right one being anterior to the left, and each lying only a little to one side of the median plane of the body. The transverse diameter of each of the testes, which as a rule is slightly shorter than its longitudinal diameter, is about one-third as great as the width of the body, their average measurements in five specimens being 0.147 mm. by 0.129 mm. The wall of the testis is an apparently homogeneous membrane, in which I have been unable to find nuclei; its cavity is ordinarily nearly filled with primitive germ cells, groups of spermatozoa, and sperm-morulæ, or groups of cells in various intermediate stages of development. The primitive germ cells do not form a continuous peripheral layer, as described by Wright and Macallum ('87) in Sphyranura osleri, but appear as numerous syncytial masses lying in the peripheral portion of the testis, as described by Looss ('85) in Distomum palliatum, and by Schwarze ('85) in Distomum endolobum. The nuclei in these syncytia may be arranged in a single layer, but commonly they form a more or less irregular group projecting some distance toward the centre of the organ. No cell boundaries are to be distinguished in these masses. The nuclei have a diameter of about 4 μ. Cells are apparently detached from these syncytia and undergo development into spermatozoa in the more central portion of the testis. Each of these proliferated cells divides, forming successively groups of two, four, eight, sixteen, and thirty-two, the cells and their nuclei becoming smaller as their number increases (Plate 2, Figs. 15–17). I believe that, as a rule, all the cells of a group divide synchronously, since all the cells of any group are very nearly equal in size and their nuclei are in the same condition. Kerbert ('81) states that the number of nuclei found in the sperm-morula of Distomum westermanni may reach six. Schwarze ('85) found as many as sixteen in Distomum endolobum, while Wright and Macallum gave the number found in Sphyranura as being over forty. Schwarze could not determine whether each nucleus of the sperm-morula was surrounded by a mass of its own cytoplasm or not; but Wright and Macallum found cells radiating from the centre of the mass, the nuclei being peripherally situated.

The large sperm-morulæ of Hemiurus crenatus (Plate 2, Fig. 18) measure about 18 μ in diameter. The first change indicative of the development of their cells into spermatozoa is seen in the nuclei, which first become oval, then wedge shaped, and later comma shaped (Figs. 18–20). Shortly after this change in the form of the nuclei begins, the cell boundaries become indistinct and at length disappear (Fig. 19). The wedge-shaped and comma-shaped nuclei have their more pointed ends directed toward the centre of the sperm-morula. The nuclei continue to elongate until finally they become thread-like and so intertwined as to resemble a dense chromatic reticulum occupying the central portion of the sperm-morula, which thus takes on an appearance very much like that of a large cell with a single large nucleus (Plate 2, Fig. 23). The intertwining nuclei at first form a nearly spherical mass, but later they appear to become arranged in the form of a shallow cup. The tails of the spermatozoa, which are outgrowths of the nucleus, in some manner break through the cytoplasm at a point corresponding with the centre of the concave surface of the cup-shaped mass, and protrude from the sperm-morula, while the heads remain within, or partly within, the cytoplasm, till all the spermatozoa have taken up this position and form a sheaf-like mass (Plate 3, Fig. 26). After the spermatozoa have detached themselves from the sperm-morula, the cytoplasm apparently assumes a nearly spherical form. These cytoplasmic remains of sperm-morulæ are quite common in the testes; their cytoplasm stains but slightly, and is apparently homogeneous, showing, however, occasionally a few small vacuoles (Plate 3, Fig. 27). Wright and Macallum state that in Sphyranura the cytoplasm degenerates, becomes fluid, and is probably partly absorbed by the developing spermatozoa.

The entire spermatozoön appears to be formed from the nucleus, though I have not been able to follow the development of the thread-like nuclei into mature spermatozoa.[1] Wright and Macallum find the entire spermatozoön to be developed from the nucleus in the species which they studied, while Schwarze, on the other hand, states that the head is formed from the nucleus and that the cytoplasm becomes transformed into the tail.

The spermatozoa apparently remain together in the sheaf-like bundles until they leave the testis. The mature spermatozoa measure 20 μ in length. The head is thread-like and 7 μ long, and is to be distinguished from the tail only by the fact that it stains a little more deeply.

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[1] In view of the results of studies on spermatogenesis in other invertebrates, as well as in vertebrates, this appearance cannot have great weight. Only extended observations, directed to this one point, on favorable material, could give trustworthy evidence on this difficult question.

Kerbert ('81) and Looss ('85) both describe peculiar semilunar or crescentic bodies which contain one or more nuclei and from which numbers of spermatozoa extend. Kerbert thinks that they probably represent sperm-morulæ in their last stage of development, and Looss is inclined to accept his explanation. Wright and Macallum found structures having a similar appearance. These are caused, they state, by a sheaf of spermatozoa being seen over a nucleus of one of the primitive germ cells, the chromatin of which is accumulated at our pole in the form of a cap. This, however, would not explain the nuclei seen in the crescents by Kerbert and Looss. The only thing I have observed that could suggest such a structure is the sperm-morula from which the tails of a *part* of the spermatozoa have escaped, the spermatozoa remaining within the cytoplasm continuing to be arranged in a cap-like mass. This would give the appearance of a crescent (Plate 3, Fig. 26). In Hemiurus there is nothing in these structures to correspond to the nuclei of the crescents, though in the forms in which nuclei have been observed it is possible that some of the nuclei of the sperm-morula remain without developing into spermatozoa. It seems possible, then, that Kerbert is correct in regarding the structures he observed as sperm-morulæ in their last stage of development, and that the trematodes in which they have been observed differ from Hemiurus crenatus in that this cytoplasm of the sperm-morula in the former takes on a saucer-like form before the tails of the spermatozoa become free from the cytoplasm and that some of the nuclei of the sperm-morula do not develop into spermatozoa.

From each testis there leads forward a very narrow duct, the *vas deferens*. The apparently structureless walls of these ducts is continuous with that of the testis. The two ducts open separately into the posterior end of the *seminal vesicle* (Fig. 1, *vsl. sem.*), which curves over the dorsal surface of the ventral sucker. The size of the seminal vesicle varies considerably, according to the number of spermatozoa which it contains. Its posterior end is usually at a point dorsal to the centre of the ventral sucker, and its anterior end often lies at a point dorsal to the pore. The vesicle may, however, extend no farther forward than the anterior margin of the ventral sucker, or it may be so enlarged as to extend to a point dorsal to the pharynx. The width of the vesicle is from one-tenth to one-third that of the body. Numerous small flattened nuclei are sometimes to be seen in its wall (Plate 3, Fig. 28), and it seems to be provided, especially in its terminal portion, with circular muscle fibres.

At its anterior end the seminal vesicle curves ventrally, turning toward the genital pore. It narrows gradually and passes by a funnel-shaped termination into the *ejaculatory duct* (Plate 3, Fig. 28, *prs. prost.*, *dt. ej.*), so that it is difficult to say where one ends and the other begins. Surrounding the narrow part of the funnel-like termination of the vesicle is a muscular mass resembling that of the pharynx and consisting largely of radiating fibres. It is possible that this structure is of service in expelling spermatozoa during copulation, and the need of something for that purpose is evident, since neither the wall of the ejaculatory duct nor that of the genital sinus appears to have well-developed muscles. Beyond the funnel-shaped part of the seminal vesicle the ductus immediately expands into an oval or spindle-shaped *pars prostatica* (Fig. 28, *prs. prost.*), the distal end of which joins the uterus to form the very short common genital duct. The pars prostatica thus constitutes practically all of the ejaculatory duct. The narrow portion joining the uterus might possibly be regarded as the ductus ejaculatorius proper.

Surrounding the passage from the seminal vesicle to the pars prostatica is a circle of cells (Figs. 28, *cl'.*) projecting into the latter. These cells are approximately conical in shape and their nuclei lie at some distance from the basement membrane on which they rest. I believe that they act as a valve, preventing the return into the seminal vesicle of spermatozoa which have been once forced into the pars.

Looss ('94, p. 184) describes similar cells at the opening of the vasa efferentia into the seminal vesicle of Distoma tereticolle. He calls them closing cells, and thinks that their function is to prevent the return of spermatozoa from the vesicle into the vasa efferentia. The wall of the pars prostatica shows several cells with large oval nuclei which project slightly into the lumen. There also project from the wall into the lumen numerous conical, or irregular, granular masses of various sizes. These are probably formed by the accumulation of secretions from the cells of the prostate gland at the openings of the ducts into the pars.

Surrounding the terminal part of the seminal vesicle are numerous pyriform or oval cells, which constitute the prostate gland. The lower end of each cell is prolonged into a slender duct, which passes downward to open into the enlargement of the ejaculatory duct referred to above as the pars prostatica. The cytoplasm of these cells is rather coarsely granular, and the nuclei, which are of an elongated oval- or spindle-shape, are always placed transversely to the axis of the cells at about their middle.

The common genital duct, which according to Looss ('96) is to be regarded as the *genital sinus*, is a short narrow tube of very nearly uniform diameter and shows no differentiation into the three parts described by Looss in Apoblema (Hemiurus) mollissimum, viz. parts like a seminal vesicle, a ductus, and a cirrus. In one specimen it was found to be partially evaginated. The wall of this part is apparently structureless, and I could not make out that it was provided with any muscle fibres.

The genital sinus is surrounded by a *cirrus-sack* (*sac. cir.*), which also encloses a very short portion of both uterus and pars prostatica. The cavity of the cirrus-sack is occupied by vesicular parenchyme.

The longitudinal muscle fibres of the body wall (Plate 3, Fig. 28, *mu. lg.*) in the region of the genital pore are broadened dorso-ventrally so as to form vertical plates or bands.

Female Sexual Organs.

The *ovarium* is situated in the median plane about one-fourth the length of the trunk from the posterior end of that part. It is broadly cordate in form, with its narrow end anterior (Fig. 1, and Plate 4, Fig. 42). Its transverse diameter, which averages about 0.16 mm., is greater than the dorso-ventral or the antero-posterior diameters, the last averaging about 0.11 mm. It is thus about one-third as wide as the body. The ovarium (Fig. 42) consists of a mass of ova of various sizes surrounded by a thin homogeneous membrane, in which I could distinguish no nuclei. The largest ova measure 9.6 μ in diameter and each has a very large nucleus and a small deeply staining nucleolus (Plate 3, Figs. 32, 33).

The short *oviduct* (Plate 3, Fig. 42, *o'dt.*) leads backward from the centre of the posterior surface of the ovarium. Its wall is somewhat thicker than that of the ovary, with which it is continuous, and in it are seen deeply staining nuclei. The oviduct is from one-third to one-half as long as the ovarium, and just anterior to, or within, the shell-gland it is joined by a duct coming from one side, the duct of the seminal receptacle. This lateral duct is similar in structure to the oviduct, but shorter.

The *seminal receptacle* (Plate 4, Figs. 37, 38, 42) is a spheroidal or oval body situated ventral and posterior to the ovarium and at one side of the median plane. The end of the duct, terminating in the seminal vesicle, widens out into a flask-shaped enlargement, which has been designated by Juel ('89) as the inner vesicle. Both the outer wall of the receptacle and the wall of the inner vesicle show occasional nuclei, which give evidence of the cellular nature of the walls. The space between the two walls is largely occupied by a mass of slightly staining granular substance, in which are always found several large nucleus-like bodies. These bodies are spherical or oval, and each shows a deeply staining spot near its centre. They are of various sizes, their diameters varying from $5.4\,\mu$ to $9\,\mu$. In some receptacles there are present large irregular particles which stain more deeply than the more finely granular substance in which they are imbedded. There are usually several more or less irregular vacuolar spaces in this substance, in which are often found numbers of spermatozoa and occasionally also yolk granules. I find, as did Juel, that the fundus of the inner vesicle is provided with a small opening (Fig. 38), which may communicate with one of these spaces or may simply be in contact with the granular substance. No *Laurer's canal* is present.

The seminal receptacle agrees very closely with that of Apoblema excisum as described by Juel, except that in neither of the walls are nuclei either described or figured by him. He interprets the granular substance between the walls, with its included nucleus-like bodies, as a plasmodium, and appears to regard the receptacle as exhibiting a rather primitive state because of its resemblance in structure to the conditions that have been described in the developing organ of other forms, where it is to a certain extent a solid body. I believe, as suggested by Looss ('94), that the granular substance consists in great part of disintegrating spermatozoa, and also that more or less of it arises from the disintegration of ova and yolk cells. The nucleus-like bodies found in the granular substance I believe to be the nuclei of ova. It is true that they are usually somewhat larger than the nuclei of the ovarian ova; but I think the increase in size is probably due, in great part at least, to the beginning of a process of disintegration. There often appears to be a small space surrounding the nucleolus. The irregular, deeply staining fragments are probably portions of yolk cells or of the cytoplasm of ova.

According to Looss ('94, p. 224) the seminal receptacle is a vesicle for the accumulation of unused spermatozoa and occasionally ova and yolk cells, which, when a Laurer's canal is present, are ultimately discharged through the canal to the exterior. In forms which do not have a Laurer's canal the elements accumulate, and, as there is no way of escape, ultimately undergo degeneration. This view explains the great size which the receptacle sometimes attains in forms which lack a Laurer's canal, and also the condition of the contents of the receptacle in such forms.

The structure which is the most difficult of explanation in connection with the seminal receptacle of appendiculate trematodes is the inner vesicle. So far as I can see, the only method by which such a structure could have arisen from a receptacle of the usual form is by an invagination of that portion of the receptacle from which the duct arises, the duct being thus carried into the interior of the vesicle. If after such invagination the terminal portion of the duct should become dilated, there would be formed a structure comparable to the inner vesicle. Should the receptaculum of appendiculate trematodes arise as the result of such a process, the space between the outer wall of the receptacle and the wall of the inner vesicle would represent the original cavity of the receptaculum, the inner vesicle would be simply an enlargement of the duct which had come to lie within the true receptacle, while the pore at the fundus of the inner vesicle would correspond to the original opening of the duct into the receptacle.

In case the receptacle of Hemiurus has arisen by a process of this kind, the wall of the inner vesicle would represent a double layer of epithelial cells. I could find no evidence that the wall consisted of two layers; but it is not much more difficult to conceive of two layers of epithelial cells being modified to form an apparently structureless membrane than it is to imagine the same change in a single layer of such cells — a modification which is known to take place, not only in the case of the wall of the seminal receptacle, but also in that of the walls of nearly all the organs of the body.

If the union between the oviduct and the duct from the seminal receptacle takes place outside of the shell-gland, the resulting duct, the *oötype*, immediately enters the shell-gland, through which it passes in a more or less horseshoe-shaped course, emerging near the anterior end of the gland. The wall of the oötype is thicker than that of any other part of the sexual organs and in it are imbedded numerous oval nuclei. It is joined near its beginning by the short unpaired part of the vitelline duct.

The *shell-gland* is a compact spheroidal mass of cells surrounding the oötype. It is situated midway between the two vitellaria, a little behind the posterior margin of the ovarium, and more ventral than either of these organs.

The *vitellaria* are two irregularly oval bodies situated just posterior to the ovarium, one on either side of, and not far from, the median plane. (Fig 1; Plate 4, Fig. 42). Their longest diameters are parallel with the longitudinal axis of the body. In cross sections of the body they are seen to occupy a dorso-lateral position. They are commonly slightly lobulated, though they sometimes have a regular oval outline.

Each gland is about one-third as wide as the body and nearly twice as long as it is wide, the average measurement of several specimens being

0.198 mm. long and 0.112 mm. wide. Each consists of a compact mass of cells of various sizes, the largest being about 14.5μ in diameter, and the average being about 7.2μ in diameter. The cells being closely packed are polyhedral, and all except the very smallest are practically filled with yolk granules. Near the centre of each cell is to be seen a spherical or slightly oval deeply staining nucleus. In the position of the glands adjacent to the opening into the ducts the cell boundaries are indistinct, and the paired vitelline ducts are often filled with free yolk granules.

One of the paired *vitelline ducts* arises from the ventral surface of each vitellarium near its mesial border and a little nearer its anterior than its posterior end. Each duct widens out somewhat where it joins the vitellarium, its wall being continuous with that of the gland. The two vitelline ducts take a direction ventral and medial, meeting directly above the shell-gland, where they unite to form the narrow unpaired duct which traverses the shell-gland to reach the oötype, with which, as before stated, it unites.

The sexual duct, upon emerging from the shell-gland, is called the *uterus;* it turns posteriad, forms two or three short transverse loops, and then a few which are more nearly longitudinal; some of these may extend to the posterior end of the trunk or even a short distance into the appendix. From this region it passes forward (Fig. 1), forming a complicated series of loops and coils, which usually occupy the greater portion of the body between the ovarium and the testes, and lie both dorsal and ventral to the intestinal coeca. In passing forward it sometimes makes a few loops dorsal to the testes, and then proceeding over the ventral sucker, joins the ejaculatory duct to form the genital sinus. The terminal portion of the uterus (Plate 3, Fig. 28) lies posterior and ventral to the seminal vesicle and ejaculatory duct. There is no great variation in the diameter of the uterus, though the portions near the shell-gland and genital sinus are smaller than the intervening portion, which is usually more or less distended with eggs. The transverse loops of the uterus ventral and posterior to the vitellaria are very often filled with spermatozoa the movements of which render the identification of this part very easy in living worms. This portion of the uterus has been designated by Looss ('94) as the *receptaculum seminis uterinum.* According to Looss the spermatozoa, which have reached this place by passing through the entire length of the uterus, are retained here for use as needed in the fertilization of eggs in the oötype; many of them, however, pass on through the oötype into the seminal receptacle, whence they are prevented from returning owing to the vibration of the cilia of the wall of the oötype. This initial portion of the uterus is more or less completely covered by a mantle of apparently undifferentiated cells (Plate 4, Fig. 42). Juel ('89) finds similar cells in Apoblema excisum, which, however, appear from his description and figure to form a more or less compact mass through which the uterus passes. He regards the cells as the remnants of the primitive meristem cells which formed this part of the sexual organs and thinks that they have remained here comparatively undifferentiated. I do not see why they could not equally well be regarded as parenchyme cells which for some reason have retained near this portion of the uterus their primitive character. As Juel states, they certainly do not appear to be gland cells. The wall of the uterus appears to be a thin structureless membrane from which nuclei can occasionally be seen projecting into the lumen. In some places I have been able to distinguish circular and longitudinal muscle fibres, which, however, are very delicate and placed some distance apart. The eggs undergo development to a considerable extent in the uterus, but on account of the included yolk it was found very difficult to follow the process satisfactorily.

Owing to lack of time and inability to obtain fresh specimens for the employment of special methods, no extended observations were made on the nervous system. Numerous sensory papillæ, similar to those described by Bettendorf ('97), were observed in the oral and ventral suckers and at the anterior end of the body. Two of the largest of these appear to be constant and are symmetrically placed at the anterior end (Fig. 1).

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EXPLANATION OF PLATES.

All figures are from preparations of *Hemiurus crenatus*. Figures 28 and 42 are diagrams reconstructed from camera outlines made from several consecutive sections. All other figures were drawn with the aid of the camera lucida.

ABBREVIATIONS.

act. or.	Oral sucker.
act. v.	Ventral sucker.
app.	Appendix.
cl.	Cell mantle of uterus.
cl′.	Closing cell.
cl. sb'cta.	Subcuticular cells.
cru. in.	Intestinal coeca.
cta.	Cuticula.
cta′.	Cuticular-like lining of anterior part of intestinal coecum.
dt. ej.	Ductus ejaculatorius proper.
dt. rcp. sem.	Duct of seminal receptacle.
dt. vt.	Vitelline duct.
fos.	Preacetabular fossa.
gl. cnch.	Shell-gland.
gl. prost.	Prostate gland.
mu′.	Parenchyme muscle fibres.
mu. crc.	Circular muscle fibres.
mu. lg.	Longitudinal muscle fibres.
mu. rtr.	Retractor muscles of the appendix.
oa.	Ovarium.
o'dt.	Oviduct.
o'typ.	Oötype.
pa'ench.	Vesicular parenchyma.
pa'ench. cl.	Cellular parenchyma.
pa'ench gran.	Peripheral granular parenchyma.
pap. sns.	Sensory papillæ.
phx.	Pharynx.
po. exc.	Excretory pore.
po. gen.	Genital pore.
prs. prost.	Pars prostatica.
rcp. sem.	Seminal receptacle.
rcp. sem. ut.	Receptaculum seminis uterinum.
sac. cir.	Cirrus-sack.
sn. gen.	Genital sinus.
te.	Testes.
trn. exc.	Excretory trunk.
ut.	Uterus.
va. exc.	Excretory vessel.
vlv. exc.	Excretory valve.
vsl. exc.	Excretory vesicle.
vsl. i.	Inner vesicle of receptaculum seminis.
vsl. sem.	Seminal vesicle.
vtm.	Vitellarium.

PLATE 1.

Fig. 1. Ventral view of a stained, cleared, and mounted specimen of Hemiurus crenatus. × 81.

Figs. 2, 3. Dorso-ventral parenchyme muscle fibres. × 1350.

Fig. 4. Spindle-shaped granular cell from the peripheral granular parenchyma.

Fig. 5. Portion of the cuticula from a longitudinal section of the body. × 1350.

Fig. 6. Cross sections of parenchyme muscle fibres. × 1350.

Figs. 7–13. Granular cells from the peripheral granular parenchyma. × 1450.

LANDER: HEMIURUS CRENATUS.

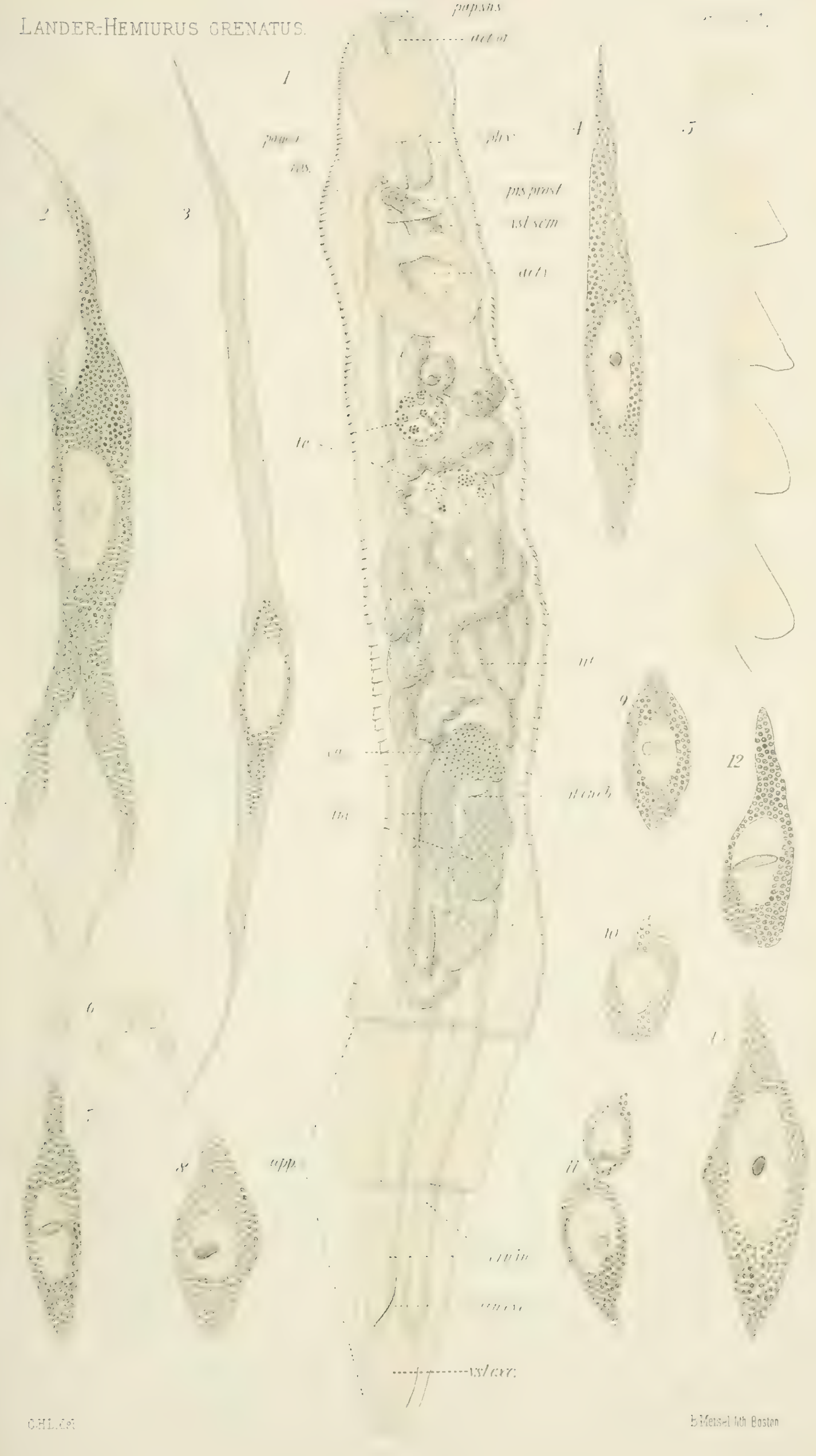
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PLATE 2.

Fig. 14. Nearly sagittal section of the posterior portion of the appendix. × 300.

Figs. 15–24. Successive stages in the development of spermatozoa, showing stages in the development of sperm-morula. × 1350.

Fig. 25. Transverse section of body through the posterior portion of the trunk. × 300.

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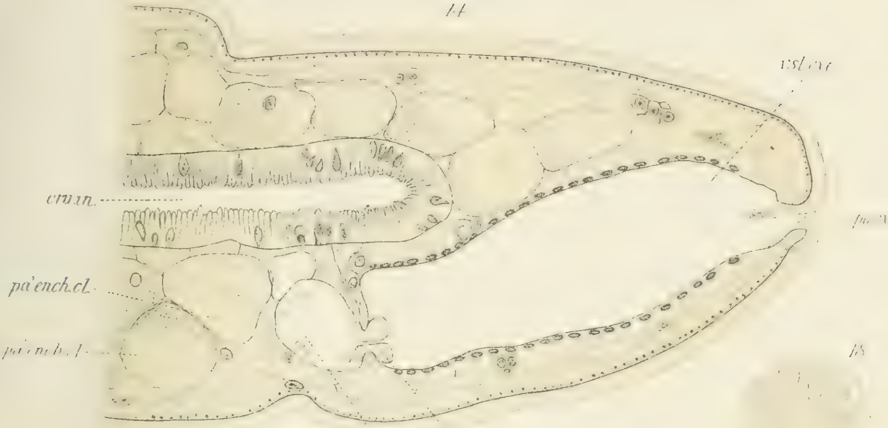
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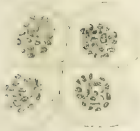

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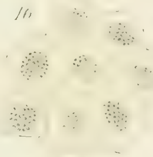

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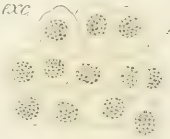

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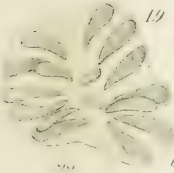

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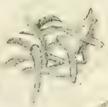

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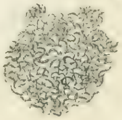

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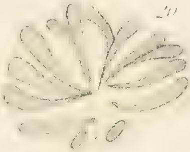

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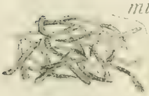

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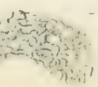

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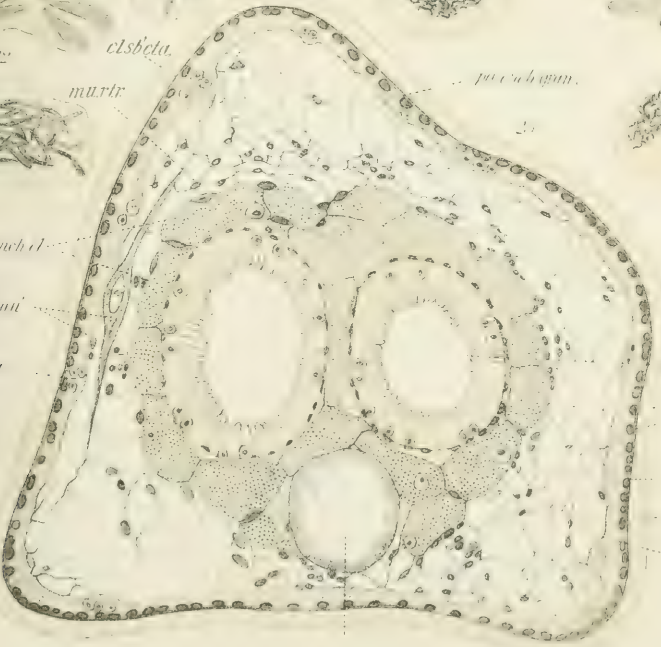
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PLATE 3.

Fig. 26. Sperm-morula. (× 1350.)

Fig. 27. Protoplasmic remains of a sperm-morula from which the spermatozoa have become detached. × 1350.

Fig. 28. Sagittal section of the terminal portion of sexual ducts, reconstructed from sections. × circa 480.

Figs. 29–31. Subcuticular cells. × 1350.

Figs. 32, 33. Ovarian ova. × 1350.

Fig. 34. Muscles of body wall from a tangential section of the body as seen from the deep surface. × 760.

Fig. 35. Section of the anterior portion of an intestinal coecum near the branching, showing the thread-like modification of the epithelial cells. Drawn from a transverse section of the body. × 480.

Fig. 36. Frontal section of a portion of an intestinal coecum, showing the deeply staining bodies between the epithelial cells. × 760.

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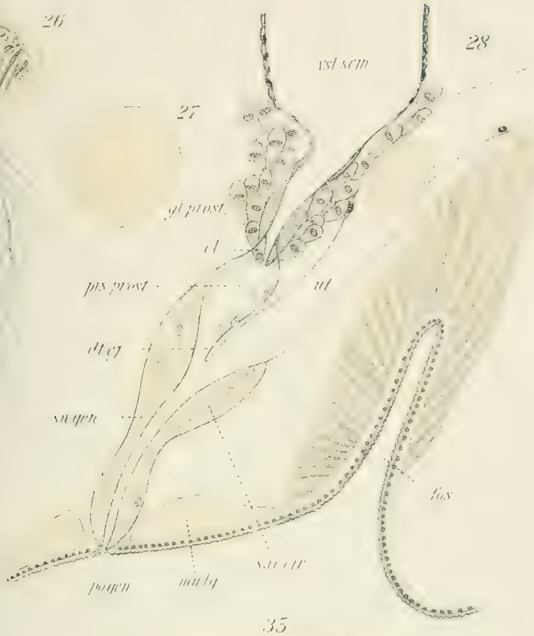
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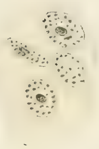

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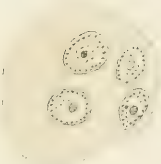

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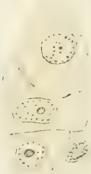

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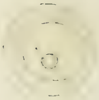

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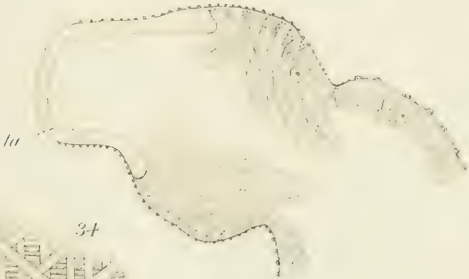

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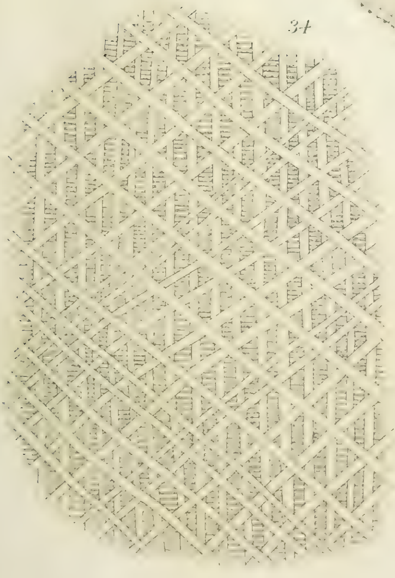

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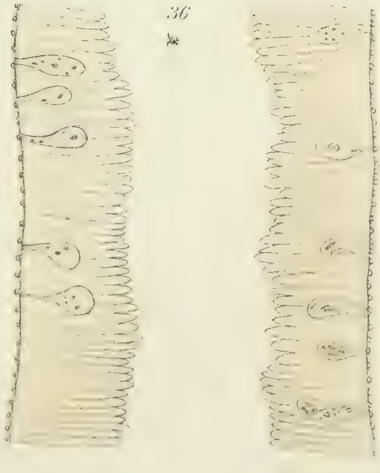

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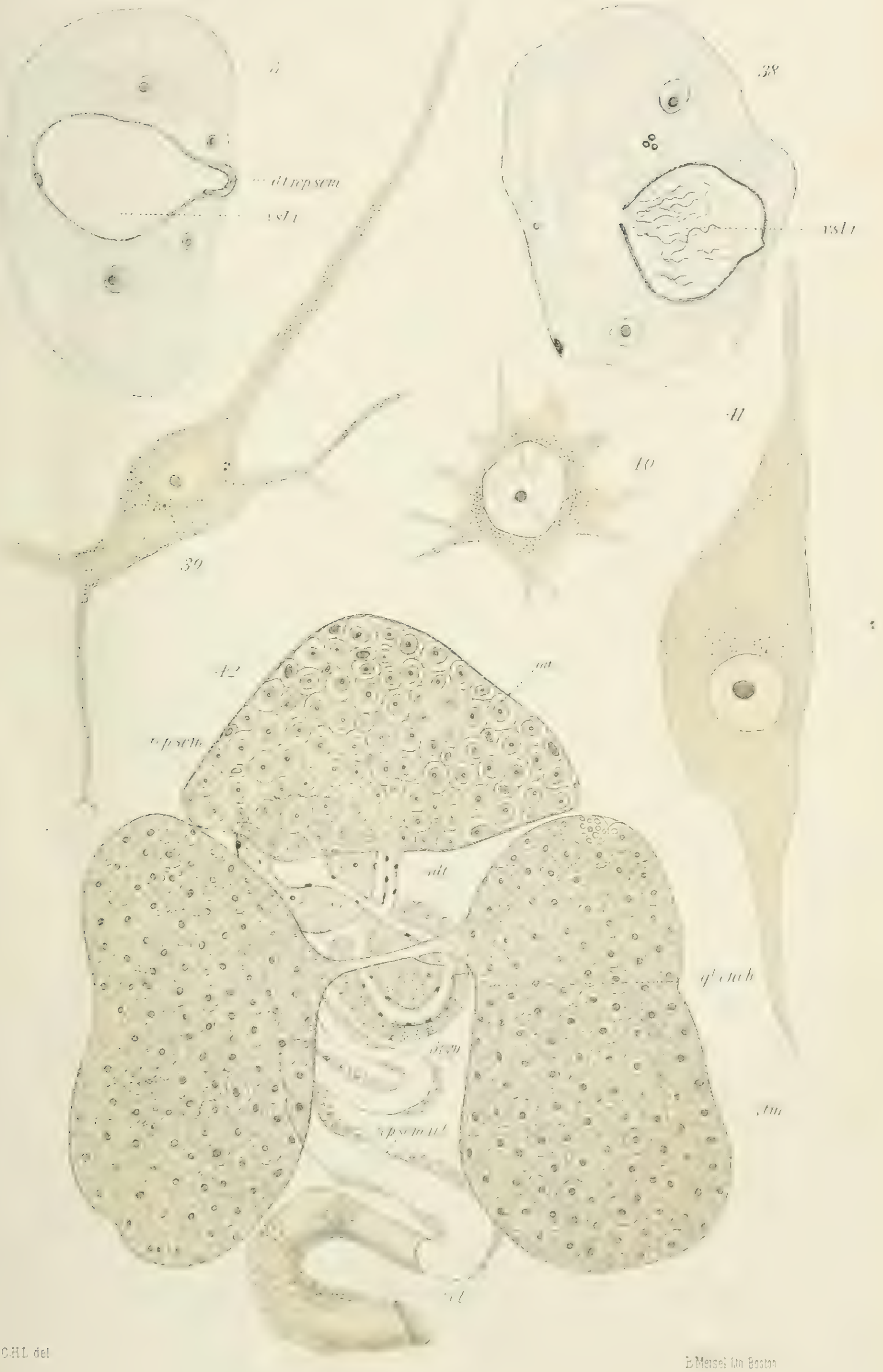
PLATE 4.

Fig. 37. Section of the seminal receptacle through the neck of its duct. × 760.

Fig. 38. Section of the seminal receptacle, showing the pore at the fundus of the inner vesicle. × 760.

Figs. 39–41. Giant cells. × 1350.

Fig. 42. Dorsal view of the female sexual organs, reconstructed from sections. The vitellaria are represented as farther apart than is actually the case in order the better to show the underlying parts. × circa 480.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Bulletin of the Museum of Comparative Zoölogy
AT HARVARD COLLEGE.
VOL. XLV. No. 2.

THE DEVELOPMENT OF THE MESONEPHROS AND THE MÜLLERIAN DUCTS IN AMPHIBIA.

BY ROBERT W. HALL.

WITH EIGHT PLATES.

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The Development of the Mesonephros and the Müllerian Duct in Amphibia.

By Robert W. Hall.

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I. Introduction.

The present investigations deal with the early development of the mesonephros and of the Müllerian duct. As these organs are in a measure independent of each other in their development, the introductory remarks on the Müllerian duct may best be left for the portion of the paper dealing with the development of that organ.

As is well known, the mesonephros consists essentially of a mass of tubules, each of which, in its typical form, may be divided into three regions: (1) a short duct leading from the body cavity into (2) a dilatation which contains an invaginated vascular mass (the glomerulus) and is in communication with the Wolffian duct by means of (3) a long, coiled duct. These three parts may together be called a *mesonephric unit*. According to their position, structure, and connections, the units fall into various sets. In the higher Amphibia two chief sets can be distinguished: a ventral set, in which the units (primary units) connect directly with the Wolffian duct, and a smaller or greater number of dorsal sets, which form connections with the Wolffian duct *indirectly* by opening into the primary set. The dorsal sets are arranged in longitudinal rows above the ventral set, and are designated as secondary, tertiary, etc.

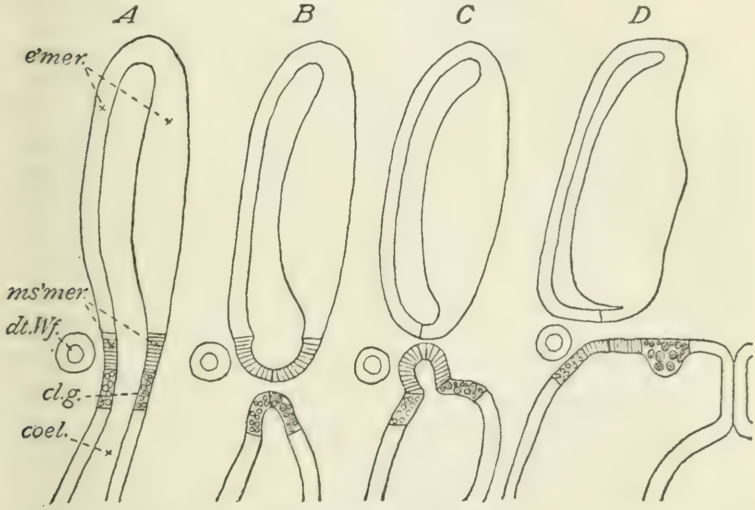
Among the problems presented by the development of the mesonephros in Amphibia three stand out at present as of greater importance than the others; it is to these three that I have given most attention. They are: (1) the origin of the tissue which forms the fundaments of the mesonephric units, (2) the origin of the dorsal sets of units, and (3) the dysmetameric condition of the primary units, — supposing them to have been originally metamerically arranged.

Before considering the views that have been held in regard to the origin of the mesonephric units, it is necessary to have a clear understanding of the condition of the mesoderm (mesothelium) at the time when they make their appearance. The mesoderm on either side of the body presents two layers, the splanchnoderm[1] mesially (ventrally) and the somatoderm[1] laterally (dorsally). These layers are separated by the

---

[1] I have coined these two words to fill a real need, the expressions "splanchnic layer of the mesoderm" and "somatic layer of the mesoderm" being awkward terms with which to designate these layers as compared with single words. The terms "splanchnopleure" and "somatopleure" have been used in this sense, but incorrectly, since the latter properly includes ectoderm as well as mesoderm and the former entoderm as well as mesoderm.

primitive coelom. While ventrally the coelom is uninterrupted, dorsally it exists in the shape of separate pockets, so that the mesoderm has the form of separate, hollow processes whose median walls are composed of splanchnoderm, the lateral walls of somatoderm. These processes are the somites. A diagrammatic cross-section of one is shown in Figure *A*. They seem to be represented in their most primitive condition in the elasmobranchs, where, according to Van Wyhe ('89), the segmentation extends ventrad even through the region of the germinal epithelium. Three regions may be more or less distinctly recognized in each somite. The most dorsal, to adopt the nomenclature of Van Wyhe, is the *epimer* (*e'mer.*, Fig. *A*), generally designated as the myotome because the main trunk-muscles are derived from it. Its cavity is the epicoelom. Passing ventrad, the next region is the *mesomer* (*ms'mer.*), enclosing the mesocoelom. The mesomer has been designated by the terms middle plate, intermediary cell-mass, "Urwirbelkommunikation," etc. The sclerotome may be considered as arising from the upper portion of its median wall, but the major part of both walls seems to enter into the formation of the nephric fundaments. For this reason



FIGURES *A–D*.

Four figures to illustrate the relation of the mesomer to the rest of the mesoderm. For explanation see Introduction (page 32). The median plane of the body is at the right in each case.

it has also been termed the nephrostome. The third, most ventral, portion of the somite is the *hypomer* and gives rise to the germ cells (*cl.g.*). These originally arose from both layers,[1] but now seem usually to be confined to the splanchnoderm. Following on the hypomer comes the unsegmented portion of the mesoderm, "lateral mesoderm," consisting of the lateral plates enclosing the true body cavity (*coel.*). It must be borne in mind that the three portions of the somite are distinguishable rather from their fate than by any early differences in shape or histological structure.

The various views held in regard to the origin of the mesonephric units may be arranged in five categories as follows: the cells composing the fundaments of the units are derived from (1) the Wolffian duct, (2) the myotome, (3) the intermediary cell-mass, (4) the peritoneal epithelium, (5) mesoderm cells of unknown origin.

The first view, that the Wolffian duct furnishes the fundaments of the mesonephric units, we may safely exclude, for there is but one group of vertebrates (Teleosts) in which such an origin has been claimed of late years (Felix, '**97**), and in this group it seems questionable not only whether the organ described is really a mesonephros, but also whether the Wolffian duct is truly homologous with that of other groups.[2]

In regard to the second, third, and fourth views, they are all reconcilable with the theory that the mesonephric tissue is always derived from a portion of the mesothelium, the mesomer, which is homologous throughout the vertebrate phylum. If the severance of the ventral (lateral) portion of the mesoderm from the dorsal portion takes place between mesomer and hypomer (Fig. *B*), the former may appear as an integral part of the epimer, and on developing give rise to the impression that the sclerotome and mesonephros arise from the myotome. If the division takes place between mesomer and epimer, the mesonephric fundament will appear either as if derived from the intermediary cell-mass (Fig. *C*), or, in case that structure has flattened out, as if from that portion of the peritoneal epithelium immediately lateral (dorsal) to the gonad (Fig. *D*). That the sclerotome never seems to arise directly from the peritoneum is due to the fact that it is given off at too early a stage.

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[1] Rabl ('**96**) states that primitive germ cells are found in the somatoderm as well as in the splanchnoderm in early stages of elasmobranch development, and I find the same condition in Amphibia.

[2] Felix states that both somatoderm and splanchnoderm enter into the formation of the Wolffian duct in the trout.

In the diagrammatic figures *A–D* it will be noticed that I have represented, in every case, the mesomer as containing tissue from both splanchnoderm and somatoderm, — thus making its cavity a true part of the primitive coelom. This important conception we owe to Adam Sedgwick ('81). Having come to the conclusion that the middle plate of the chick represented a portion of the splanchnoderm and somatoderm, he proceeded to verify it in the elasmobranchs.

The condition he described in these animals is briefly this: The middle plate is in the form of a tube connecting the coelom of the lateral mesoderm with the cavity of the myotome. This tube becomes cut off from the myotome and its dorsal end curves downward and outward[1] to join the pronephric (Wolffian) duct.[2] At a point near its connection with the lateral plates, the dorsal wall of the tubule is invaginated to form a glomerulus. The portion between the Malpighian body thus formed and the lateral plates is the outer tubule; that between the Malpighian body and the duct is the inner tubule. The nephrostome is thus formed by the persistence of an opening already present, and the cavities of the outer and inner tubules, as well as that of the Malpighian body, are a portion of the primitive body cavity.

I believe Sedgwick's description is correct except in one important detail; instead of the inner tubule arising by a bending downward of the blind pocket, which — as it contains both splanchnoderm and somatoderm — would cause the lumen of the inner tubule to represent primary coelom, the inner tubule is formed *entirely by means of the evagination of the somatic layer.* This would make the lumen of the inner tubule, in a way, a secondary one. Field ('91) lays stress on this point, and it has been vaguely recognized by other authors.

Sedgwick's conception, with more or less modification, has been found to apply to most of the vertebrate groups which have been studied since 1881. In the Amphibia, however, two of the authors who have expressed an opinion on the subject since that date have derived the mesonephric elements from evaginations of the peritoneum.[3] Hoffmann ('86) describes their origin in Triton and states that the serial evaginations (in the form of solid outgrowths) retain their connection with the peritoneum to form the nephrostomes. Field ('91), in a work on the pronephros of Amblystoma, simply mentions that he could not be certain as to the mode of origin of the mesonephric elements, but is inclined to think that they are derived from the peritoneum.

---

[1] A glance at Figure *C*, page 33, may aid in understanding this process.

[2] No distinction will be made in this paper between pronephric and Wolffian ducts.

[3] I leave out of account, for the present, that primitive amphibian, Ichthyophis.

As was pointed out above, such an origin of the mesonephric units would not be incompatible with the existence of a mesonephric fundament (the mesomer) homologous throughout the vertebrate series, and would even seem to be fairly well established for Petromyzon (Wheeler, '99). The *fact* of such an origin in Amphibia has, however, been disputed by Marshall and Bles ('90). Although they were unable to decide as to the true origin of the mesonephric fundaments, they were certain that these do not arise from the peritoneum and that the nephrostomes join them secondarily.

The first problem, then, is that of the origin of the mesonephric fundaments and the question as to whether or not both layers of the mesoderm enter into it.

The origin of the dorsal sets of units seems never to have been carefully investigated. Spengel ('76) thought they arose by a splitting of the Malpighian body of the primary units. Fürbringer ('78) states that their origin is similar to that of the primary, which he describes as developing (at least in the case of the more anterior units) from solid peritoneal outgrowths. Hoffmann ('86) came to the conclusion that they are derived from the primary units because they equal them in number.

Material and Methods.

I have no reason to doubt that all of the urodele material on which the investigation of the mesonephros is based consists of Amblystoma punctatum Linn., as that is the only species of Amblystoma at all common about Cambridge, Massachusetts, where the eggs were collected. The male and female animals of this species were often found in the ponds where the eggs were collected. Two females of the species named, when brought home, laid freely in captivity; and the developing embryos and larvae differed in no respect from any of those collected from the ponds. The large translucent masses of jelly containing the eggs are attached to twigs and rushes about a foot beneath the surface of the water. In the near neighborhood, the white, fungus-like spermatophores can generally be seen dotting the twigs and leaves of the bottom. The eggs are laid as soon as the ice is well out of the ponds.

The Amblystoma material which was used in the study of the Müllerian duct was mostly collected at New Haven, Connecticut. It consists of larvae in their second year. The majority were caught early in May and raised in captivity. Although well fed, these were shorter by about ten millimetres at the time of their metamorphosis than those which were not kept in captivity. This difference in size is already observable at the time when the Müllerian duct begins to develop. For this reason the length of the specimen has little significance. In the case of animals raised in captivity the fundament of the duct first appears when they have reached a length of thirty-five millimetres; in the others, when they are about forty-five millimetres long.[1]

Some of my specimens were given me by Professor J. S. Kingsley, who also loaned me some of his slides. I wish here to express my gratitude for his kindness, and that of Doctors F. D. Lambert, H. V. Neal, and J. H. McGregor, who also put their material at my disposal.

My anuran material consists of specimens of Rana sylvatica Le Conte, which were all raised from the egg. The eggs of this species can be distinguished from those of any other New England anuran by the early date at which they are laid. They are deposited quite as early as those of Amblystoma punctatum and are often found with the eggs of that species in cold shaded pools, where they are attached in a similar manner to twigs, etc. The jelly in which they are imbedded forms rounded masses of a tougher consistency than the egg masses of any other New England frog. The development of the Müllerian duct was also investigated in Hyla versicolor Le Conte. The larvae were collected at Wood's Hole, Mass., in July and kept in captivity. They are distinguished from other tadpoles by their crimson tails and blunt snouts.

I tried a number of killing reagents. The most serviceable one for the younger stages seems to be a saturated aqueous solution of corrosive sublimate with five per cent acetic acid. Kleinenberg's picro-sulphuric mixture gave good results when the embryos were imbedded in paraffin soon after hardening. By far the best results with embryos from ten millimetres up were obtained with Zenker's fixing reagent. Unfortunately this was not learned early enough to try it on younger stages. Felix found it unsuitable for trout which still had the tissues loaded with yolk and this might also prove to be the case with amphibian material. I kept some of the hardened embryos in 82 per cent alcohol, some in 90 per cent. After five or six months I was much chagrined to find that none of the material so preserved except the older larvæ was of any value for finer details. I hear from various sources the same report in regard to the early stages of amphibian material and conclude that the only safe way is to imbed and preserve in paraffin. Much difficulty was encountered at first in cutting the younger specimens. The yolk crumbled under the knife and utterly destroyed the tissues. This trouble later disappeared. I believe the change in method which brought about the amelioration was a shorter sojourn of the objects in the higher grades of alcohol, xylol, and paraffin. I generally found it sufficient to leave the smaller embryos but five minutes in each of the following: absolute alcohol, xylol, soft paraffin, hard paraffin. A number of stains were tried. I finally restricted myself to Delafield's haematoxylin followed by orange G. A weak solution of Delafield's haematoxylin in water was employed, of such a strength that the nuclei stained to the proper shade in about thirty minutes (staining was always done on the slide). Treated in this way this stain is so selective that no decolorizing is necessary. The slides were then washed in tap-water and passed through ascending grades of alcohol, in one of which (preferably 70 per cent) some crystals of orange G had been dissolved. The latter does not overstain the yolk, as most plasma stains do. This process makes the nuclei blue, in sharp contrast with the orange yolk and paler yellow cytoplasm. In the older stages the cytoplasm usually stains a faint blue.

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[1] Thinking that I might be dealing with two different species, I took one of the smaller larvae which had metamorphosed to Mr. Samuel Garman. He tells me that he sees no reason to doubt that it is a specimen of Amblystoma punctatum.

In describing the mesonephric development I shall make use of the following terms: blastulae (the "Bläschen" of German writers), inner tubule (main tubule, canalis principalis), inner funnel, outer tubule or nephrostomal tubule (canalis nephrostomalis), outer funnel or nephrostomē, Malpighian body, and glomerulus. The "visceral layer" of the Malpighian body (Semon) I shall call the glomerular covering, reserving the term "Bowman's capsule" for the "parietal layer."

The work was begun and the part relating to Amblystoma practically completed at the Museum of Comparative Zoölogy of Harvard University at the suggestion and under the direction of Dr. E. L. Mark. It gives me pleasure to express my gratitude to him for his constant aid. I wish also to thank Doctors W. E. Castle and G. H. Parker for their interest and suggestions. The work was completed at the Sheffield Biological Laboratory of Yale University, and I am indebted to Professors S. I. Smith and W. R. Coe of that institution for many favors.

II. Development of the Mesonephros.

A. Amblystoma.

Stage I.

In embryos of Stage I, the head and tail are already slightly differentiated from the oblong mass of the body. The eye is visible, although sections do not show any sign of the lens.

About nine body somites are already established, — two in front of the pronephric thickening, two in the region of that thickening, and five posterior to it. In order to make my account more readily comparable with that of Field ('**91**), I shall call the somites in connection with which the pronephros is developed somites three and four, ignoring the head segments.

In cross-sections the somites have in outline the form of triangles (Figure 1, Plate **1**), the median sides of which abut on the neural tube and chorda. In the middle of the somite there is a lumen. The walls are thick and composed of a single layer of slender columnar cells heavily pigmented in the ends bordering on the lumen. From Figure 1 it will be seen that the lateral plates (*so'drm. l.* and *spl'drm. l.*) are considerably thinner than the walls of the somite, and that the lateral extension of the lumen of the somite between them is indicated by cell-boundaries only, there being, generally, no real cavity. On following the lateral plates ventrad they are seen to become still thinner.

Frontal sections show the somites as rounded rectangles, somewhat elongated laterally and flattened against each other. Segmentation of the mesoderm extends as far laterad as the point *nph'tm.* in Figure 1; that is, to a point about on a level with the dorso-median boundary of the Wolffian duct (*dt. Wf.*). The only clue to the position of the mesomer is the pronephric thickening, which, as it is the fundament of the pronephric tubules, must lie in the mesomeric somatoderm. The section from which Figure 1 was taken passed through the seventh somite. Superposition of camera drawings of that section and those anterior to it shows the pronephric thickening to occupy a position corresponding to that portion of the somatoderm marked *nph'tm.* in Figure 1. How much of the ventral (splanchnic) wall of the somite is to be assigned to the mesomer, is not to be determined at this stage. The Wolffian duct has reached the middle of the eighth somite.

Stage II.

Embryos of Stage II have increased considerably in length over those of Stage I. The head and tail are clearly marked off from the body. The optic vesicles still communicate widely with the forebrain, and there is no sign of a lens-thickening.

The chief difference in the structure of the somite between this and the previous stage, as may be seen from Figure 3, is a great increase in its dorso-ventral diameter. Its lumen in that diameter has increased even more than has the somite, but it has decreased in its antero-posterior diameter, so that the anterior and posterior walls of the somite now almost touch each other. Figure 3 does not fully show this increase in the vertical diameter of the lumen, because the somites are oblique both to the frontal and slightly to the sagittal plane of the body, their anterior and posterior walls sloping backward both dorsally and laterally. Sections in the three planes, transverse, sagittal and frontal, show the anterior, posterior, and median walls of the somites to be thick, the ventral and dorso-lateral walls thin. The somites are more closely applied to each other than in the preceding stage, and fit together like opisthocoelous vertebrae. The lateral mesoderm is more distinctly differentiated from the somite than in Stage I.

The duct has reached somite 14. *Germ cells* are present and rather conspicuous opposite somite 11. They are differentiated out of the *most dorso-median portion of the lateral mesoderm*[1] by the increase in size of cells of *both splanchnoderm and somatoderm* (*cl. g.*, Fig. 5, Plate **1**). These cells form a rod parallel with the lateral edge of the somite and enclose a small lumen which is a portion of the coelom. They are still connected with the rest of the lateral mesoderm and with the mesomer. At *va. sng.* (Figure 3) are seen cells which apparently give rise later to blood-vessels. Their origin is unknown.

Stage III.

The embryos of Stage III possess about twenty somites and measure from 5 to 5.5 mm. in length. The fundaments of the gills are beginning to show as external protuberances. The cavity of the optic vesicle is nearly obliterated, and the lens-thickening is beginning to appear.

A comparison of Figure 13 (Plate **2**), with Figure 3 shows the main points of advance in the development of the somite between this stage and Stage II. The changes will be seen to consist in an alteration in shape and in an increased difference in the relative thickness of the walls. In Figure 3 it will be noticed that the lateral wall is bent outward to a slight extent just above the duct (at the point marked *so. v-l.*). In Stage III this bend is so pronounced that the junction of mesomer and lateral plates (Figure 13, *nph'tm.*) lies ventral to the edge of the somite. The ventral wall of the somite thus contains a small amount of somatoderm. By the increased thickening of the anterior, posterior, and median walls, the cavity of the somite has become nearly obliterated. There is left only a narrow fissure-like lumen extending from the ventro-median to the lateral and thence to the dorsal angle.

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[1] This tissue represents, of course, the hypomer, but segmentation is rather indistinct and very transitory.

One of the most important points to be noticed in this stage is the beginning of the differentiation of the sclerotome, the first indication of which may be seen in Figure 13, in the slight extension (*scl'tm.*) of the ventral wall of the somite toward the chorda. The tissue which forms the sclerotome is certainly derived from the splanchnoderm of the ventral wall of the somite, but at precisely what point or points the cell multiplication takes place, I have been unable to determine. The peculiar form and arrangement of the cells of the median portion of this ventral wall (see Figure 13) suggest, however, that this cell-layer is being shoved mediad, and I suspect that the increase of cells takes place quite near the point of union of mesomer and lateral plates, perhaps near the point a. However this may be, I think we are justified in considering all of the splanchnoderm of the ventral wall *lateral* to the point a, as belonging to the mesomer. The mesomer thus consists of a splanchnic portion, just described, extending mesiad from the point of union with the laterad mesoderm at least as far as the point a, and a somatic portion, which, beginning at the point of union with the lateral mesoderm, extends laterad toward the outer angle of the somite (see the discussion of the position of the pronephric thickening in the description of Stage I).

The Wolffian duct at Stage III has joined the cloaca opposite the anterior end of somite 19. In the anterior half of the embryo it already possesses a small lumen.

Germ cells are conspicuous from the posterior end of somite 11 to the posterior end of somite 15. They are most conspicuous intersegmentally, but extend a short distance beneath the two adjacent somites. In the last segment in which they are seen (somite 15), they extend along the entire somite. Although partially forced into an intersegmental position, the germ-cell masses do not lose their connection with the mesomers.

The loose tissue shown at *va. sng.* in Figure 3 has in this stage developed into the walls of blood spaces (Plate **2**, Figure 13, *va. sng.*), which as yet contain no corpuscles.

Stage IV.

This stage may be considered rather briefly. The embryos measure from 6 to 6.5 mm. in length. The deeper layer of the ectoderm over the optic vesicle has invaginated to form a conical lens-thickening of high columnar cells.

The cells of the splanchnic layer of the epimer have increased in number and are beginning to take on the form of muscle cells, but there is no indication of fibrillae.

Figure 2 (Plate **1**) shows the main features to which I wish to call attention. These are the further migration of the duct (*dt. Wf.*) inward under the somite, and the growth of the sclerotome (*scl'tm.*), which has now extended dorsad between epimer and neural tube and encountered the sclerotome of the opposite side of the body. Loose cells of unknown origin lie where the aorta later appears and presumably contribute to its formation. Similar scattered cells are commonly found beneath the whole of the ventral wall of the somite, where they later become much more numerous. In sections posterior to the one figured, the lumen of the somite (*coel. so.*) extends up to its dorsal angle.

Stage V.

I take as a type of this stage an embryo 9.25 mm. in length. A considerable advance over the last stage has been accomplished in the specialization of tissues. The main mass of the epimer is composed of longitudinally directed muscle cells whose nuclei occupy a central position. In frontal sections the non-nucleated ends of the cells are seen to form broad, clear bands at either end of the somite. The clear bands of successive somites are separated by a thin layer of connective tissue, the myocomma (Plate **1**, Fig. 7, *my'cm.*, and Fig. 9). Muscle fibrillae are clearly distinguishable. The sclerotome has only occasionally the form of a continuous layer of cells, having been for the most part transformed into finely branching, mesenchymatous tissue with scattered nuclei, which fills the space between the somites, the neural tube, chorda, etc. Between the ventro-median side of the somite and the entoderm the sclerotome seems to retain some of its continuity with the somite, but how much of that tissue (Fig. 8, β) is sclerotome and how much has arisen from the loose cells mentioned in the description of

Stage V it is impossible to decide. The more dorsal portion of the somatic layer of the epimer has also been converted into mesenchyme. Of the two thinner walls of the somite, the lateral and the ventral, there thus remain only the mesomer and a small adjacent part of the somatic layer of the epimer (*so'drm. t.*, Fig. 7). The latter bounds the ventral and lateral sides of the remnant of the epicoelom.

To understand Figures 7, 8, and 9, it is necessary to bear in mind the opisthocoelous character of the somites and their oblique position. Not only are they placed obliquely, but their ventro-lateral portion, with the enclosed lumen, has been stretched backward, so to speak, until the most posterior point of each somite lies well back on the next following. Figure 8 represents a section through the middle of a somite; Figure 7 shows one passing back of the middle of one and barely cutting the anterior end of another (which would be seen above and to the right of the myocomma, *my'cm.*); the section represented in Figure 9 passes through the posterior end of a somite. The overlapping of the somites causes the mesomer to extend obliquely outward and backward, so that in the anterior portion of the somite it lies some distance from the duct and mesad of the postcardinal vein, while toward the posterior end it lies against the duct (Fig. 9, *ms'mer.*).

The point to which attention is to be especially directed is the appearance of the mesomer, for it is in the mesomer and at this stage that the *fundaments of the mesonephros* are first discernible. These fundaments arise in the embryo under consideration in the posterior fourth of each mesomer from somite 9 to somite 18, inclusive. Without doubt the one or two remaining somites anterior to the cloaca give rise in a similar manner to mesonephric fundaments, as the blastema[1] is seen to extend in older larvae to the posterior end of the duct. This question could not be decided definitely, as that part of the body in the particular embryo described above was lost, and it was found that in other specimens (and presumably this would have been likewise true of that specimen) the curving of the body renders all processes in the posterior region obscure.

The differentiation of the mesonephric fundaments may be described as follows: the posterior fourth of each mesomer (Figs. 7, 9, *ms'mer.*) distinguishes itself from the remaining portion (and from the *entire* mesomer of somites anterior to the ninth) by three peculiarities: the crowding of the nuclei, the intensity of the nuclear stain, and the presence of a large amount of pigment throughout the cells. These three peculiarities combine to give these earliest fundaments of the mesonephros that conspicuousness which characterizes the organ until it becomes functional.

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[1] The term "blastema" has been employed to designate the continuous cell-mass from which the mesonephros arises in Amniota. As will be shown in the description of the next stage, the term applies equally well to Urodela.

That both somatic and splanchnic layers of the mesomer participate in the formation of these fundaments seems unquestionable from the appearance of such sections as that shown in Figure 7, where two groups of nuclei will be noticed, one on each side of the point marked *ms'mer.* Although such a favorable fundament (that is, one showing a clear separation into two masses, a somatic and a splanchnic) is rather rare, I have seen, nevertheless, such a condition often enough to justify the belief that it is not without significance.

The only additional points to be noted in this stage are the presence of a distinct aorta (Fig. 7) and postcardinal vein (*va. sng.*, Fig. 8), the open lumen of the duct, and the coalescence of the germ-cell masses to form a more or less continuous ridge, no longer showing any sign of segmentation. This ridge extends from the posterior end of somite 10 to somite 16 (see Diagram 1, page 56, column *A*).

The earliest traces of the mesonephros thus appear as specialized masses of cells differentiated from the posterior portion of the mesomers from somite 9 back to, presumably, the posterior end of the duct. The fundaments are metameric, there being one for each segment. They are derived from both splanchnoderm and somatoderm.

Stage VI.

The larva described as illustrating this stage measured 10 mm. in length. The development of the mesonephric fundament is very rapid in larvae from 9 to 10 mm. long, which explains the fact that among the many specimens sectioned, I have been able to find but one good example of each of the Stages V. and VI.

Figures 10, 11, and 12 (Plate 1) illustrate the changes which the somites have undergone. The mesomer, duct, and dorso-median angle of the lateral mesoderm have migrated still farther mediad. The remaining portion of the somatic layer of the epimer has been more or less completely converted into mesenchyme, thus obliterating the remains of the epicoelom by depriving it of one of its walls. In the section represented by Figure 12 this process has been nearly completed. In that shown in Figure 10 it is still in progress, the outer wall being very thin. That portion of the somatoderm directly over the duct has also become very thin, or has entirely disappeared. Occasionally, however, as at *so'drm. t.*, Figure 12, this layer is still visible as a thin membrane connecting the mesomer with the *lateral* portions of the epimeric somatoderm. Aside from this slender connection, the mesomer has been completely severed from the overlying epimer. The all-important change to be noted is the *fusion of the successive mesomers to form a continuous cord.* That portion of the cord extending from somite 9 to somite 19 or 20 is the *mesonephric blastema.* This blastema is not of uniform diameter throughout its length, but presents swellings extending from the posterior third of each somite backward to a point opposite the anterior third of the following one. These swellings present all the characteristics of the mesonephric fundaments described in the preceding stage, and are presumably identical with them. That each has moved slightly backward from the position in the posterior part of each somite where it arose, thus bringing its greatest diameter into an intersegmental position, is not strange, as the mesomer is now practically free from the rest of the somite. Cross-sections of two of these swellings are shown in Figures 10 and 11 (*fnd. ms'nph.*). In Figure 10 may still be seen a condition suggestive of an origin from the two layers, splanchnoderm and somatoderm (compare Fig. 7).

Opposite the middle of each somite, from the ninth to the twelfth, there is an additional swelling which is quite evident, though much smaller than the ones just described. Opposite somite 13 there seem to be at least two of these smaller thickenings.[1] For convenience I shall refer to all of these smaller swellings as swellings or units of the "*second order*," to the larger ones as those of the "*first order.*" Figure 12 (*fnd. ms'nph.*) shows a cross-section of one of the most distinct of the former, that opposite the middle of the twelfth somite. The units of the second order are probably developed from the tissue of the anterior portion of the mesomer just before or shortly after the fusion of the mesomers to form the continuous blastema.

A word should be said in respect to the relation of the blastema to the lateral plates. In Figures 11 and 12 there is shown an intimate fusion between the blastema and the lateral plates, here represented by the germ-cell mass. This is true only as far forward as the germ-cell mass extends, — to somite 11. There are about six thickenings anterior to that point, which seem in this larva to have no connection with the lateral plates.

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[1] Unfortunately I do not possess the portion of this larva back of somite 13.

Stage VII.

The larvae of this stage measure from 10 to 10.5 mm. in length. The duct, the median angle of the lateral mesoderm, and the mesonephric blastema have migrated still farther mediad, so that the blastema now lies at the ventro-median angle of the epimer. The most noteworthy change is the transformation of the thickenings of the blastema into more clearly cut masses, which from this stage on I shall call *blastulae* (the "Bläschen" of German authors). These, although still elongated antero-posteriorly, are shorter than in the preceding stage and more rounded in cross-section. Their nuclei have begun to place themselves radially about the long axis of the blastula, leaving a clear protoplasmic area in the centre. The first indication of this arrangement is to be seen in Plate 2, Figure 17, *fnd. ms'nph.* As will be seen in columns *C* and *D* of Diagram 1 (page 56), the blastulae are distributed as in Stage VI (column *B*, Diagram 1), one of the first order and one of the second order for each segment. As before, this statement is true only for the more anterior somites. From somite 13 or 14 caudad those of the second order become more numerous. Those lying in the regions where the germ-cell mass exists are in close contact with it. The fact that those anterior to it are invariably connected with the lateral plates makes it doubtful whether the separation mentioned in the preceding stage was artificial, or the continuity in the present stage is due to a secondary fusion.

Posterior to somite 15 the blastema becomes more slender and more uniform in diameter. Although there are occasional swellings in it, they are ill defined and irregularly placed, and may possibly not represent blastulae.

Stage VIII.

The larva on which I base my descriptions of Stage VIII measures 12 mm. in length. Figure 19 (Plate 2) represents a portion of a cross-section through the posterior end of somite 11 of both right and left sides, and cuts a pair of blastulae near their centres.

The epimers, as the result of the great increase in their dorso-ventral diameter, now project far below the chorda, and those of the opposite sides of the body have approached each other so that they now compress the aorta slightly. In this stage the body cavity (*coel.*) is represented by an actual lumen immediately ventral and lateral to the germ-cell mass, the lumina of the opposite sides of the body being separated by a thin layer of tissue, the mesentery.

The mesonephric blastulae are very conspicuous, being considerably larger than in Stage VII. They are more rounded in cross-section, and the radial arrangement of the nuclei, which began to be noticeable in the last stage, is well established. This radial arrangement is seen not only in cross-sections, but in frontal and sagittal sections as well, which proves that the nuclei all point toward a common centre. In frontal sections the blastulae are seen to be elongated masses, two or three times as long as they are broad, each one just touching its neighbors. While the median wall of the blastula appears nearly straight and is parallel with the long axis of the body, the lateral wall appears quite convex, projecting outward over the Wolffian duct. *This wall is also thicker than the median* and has more elongated nuclei.[1]

It is a point of importance that in this stage the enlargements which I have termed "blastulae of the second order" are no longer to be distinguished by their size. They have overtaken those of the first order in their development, and the two sets are henceforth indistinguishable except by their position, and even that criterion does not long serve to distinguish them.

The Dorsal Extent of the Somatic and Splanchnic Layers of the Lateral Mesoderm.

Before proceeding further with the description, I wish to make a slight digression to discuss the question of the dorsal extent of the somatic and splanchnic layers of the lateral mesoderm. The study of such a stage as that represented by Figure 19 would lead one to think that the junction of the two layers lay directly over the point marked *coel.* It really lies a little mesad of the duct, at the point designated *nph'stm.* In order to justify this statement it is necessary to revert to the description of Stage II. It was there shown that the germ cells were differentiated from both layers of the lateral mesoderm in the region of their junction with the corresponding layers of the mesomer. In all of the later stages the mesomer (represented by the mesonephric blastema or blastulae) has remained closely connected with the germ-cell mass and has migrated with it toward the median plane of the body. As the morphologically dorsal angle of the body cavity must be at the junction of mesomer with lateral mesoderm, it lies, in such a section as that represented by Figure 17, *in the germ-cell mass* near the point *nph'stm.* How, then, has the apparent dorsal angle in Figure 19 (*coel.*) arisen?

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[1] This characteristic, so noticeable in frontal sections, is shown only to a slight extent in the particular cross-section figured (Fig. 19).

The arrangement of the nuclei at the point *coel.* in Figure 17 seems to indicate that it has arisen as an outfolding of the splanchnic layer of the lateral mesoderm toward the median plane. In the elasmobranchs such an outfolding is very evident (Rabl, '**96**, Taf. 15, Fig. 8 ; Rückert, '**88**, Taf. 15, Fig. 21). In those animals the mesonephric nephrostomes unquestionably mark the morphologically dorsal angle of the body cavity, and they lie just mesad to the duct, while the apparent dorsal angle is much nearer the median plane, and is formed by an outfolding of the splanchnic layer of the lateral mesoderm.

In Figure 19 the ventral angle of the blastula (directly above the point *nph'stm.*), which is to form the nephrostome, seems to have moved ventrad into a position between the duct and the germ-cell mass. Such a shifting of the fundament of the nephrostome would seem to necessitate a severance of its connections with the two layers of the lateral mesoderm and the re-establishment of a connection exclusively with the somatic layer of the lateral mesoderm. Such a condition would exclude a strict parallel between the nephrostome development in Amblystoma and that in elasmobranchs, where, as above stated, the original connection between the lumen of the mesomer and that of lateral plates is retained as the nephrostome, which consequently opens at the point of union of the two layers of the lateral mesoderm. But such an interruption of the connection between the fundament of the nephrostome and the lateral plates in Amblystoma is not the only possible interpretation of the condition shown in Figure 19. The indicated migration of the ventral region of the blastula, *without loss of continuity between the layers of the mesomer and those of the lateral plate* might be brought about by a degeneration of the germ cells of the somatic layer into ordinary epithelial cells. Rabl ('**96**) assumes precisely such a degeneration in elasmobranchs, where, in early stages, he finds germ cells present in *both* layers, whereas later they are confined to the splanchnoderm. In Amblystoma the degeneration of the cells in question is rendered probable not only by the fact that *all* of the germ cells disappear from posterior somites (see Diagram 1), but also by the additional fact that, in the larva from which Figure 19 was taken, there were frequently seen, lying just ventral to the blastula cells, which are far too small to be germ cells, yet resemble them in containing a conspicuous amount of yolk.

There is probably, then, a persistent connection between the two layers of the blastula and the corresponding layers of the lateral mesoderm, which brings the condition in Amblystoma into close conformity with that in elasmobranchs, and, what is of even greater importance, with that found by Semon ('92) in Ichthyophis. (See Figures *F* to *H*, page 72.)

What has been said in regard to this persistent connection applies only to the somites anterior to the sixteenth. Posterior to the sixteenth the blastulae are separated more and more widely from the peritoneum by loose, mesenchymatous tissue (compare Figure 20, a section through somite 16 of a larva 21 mm. long). The nephrostomes of these posterior units, as well as those of all secondary units, must therefore join the peritoneum secondarily. Whether those of the posterior primary units join the peritoneum at the point of junction of somatic and splanchnic layers or not, is impossible to determine. It is certain that these and the nephrostomes of secondary units cannot both open at the line of junction, for both sets of nephrostomes are often seen in one cross-section, opening at some distance from each other (see Fig. 24, Plate 3, *nph'stm. 1* and *nph'stm. 2*).

Development of a Mesonephric Unit in Amblystoma compared with that in Pristiurus.

In resuming the description of the mesonephros I shall no longer describe larvae at successive stages of development, but instead briefly describe the *development of a blastula* into a functional unit,[1] — briefly, because these changes have been well described, in the main, by Fürbringer ('78) and others.

In order the better to compare the course of development in Amblystoma with that in elasmobranchs, it will be well to trace the history of such a unit in Pristiurus as described by Rabl ('96). According to that author the blastula is formed in the following manner. The mesomer, which is in the form of an epithelial tube connecting the lateral plates with the epimer, is early rendered incomplete by the breaking up of the dorsal portion of the splanchnoderm to form the sclerotome. The gap thus formed is again closed by the growth mesad and ventrad of the dorsal edge of the somatoderm, which has already freed itself from the epimer. There is thus formed a blind pocket opening into the coelom. Of the median wall of this pocket, a portion adjoining the lateral mesoderm is thus formed of splanchnoderm. The rest of the pocket is composed of somatoderm. The splanchnic portion is thin; the somatic portion, especially on the lateral side of the blastula, is thick. The pocket then becomes differentiated to form a wider portion distally and a narrower portion proximally. The latter becomes the outer tubule, while from the much-thickened lateral wall of the former the inner tubule is differentiated and grows outward and downward to join the duct. The median wall, or sometimes the dorsal wall, of the distal portion becomes invaginated to form the Malpighian body.

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[1] The development can be followed by examining successive units in the same larva, beginning at the caudal end of the series, where they are less differentiated, and progressing cephalad.

As far as we have progressed in our description of the development of a unit in Amblystoma, it will be seen that there is a rather close similarity to the development in Pristiurus. As will be remembered, the blastula in Amblystoma is composed of cells from both splanchnoderm and somatoderm. These layers probably retain their original relative position, the somatoderm giving rise to the thick lateral wall of the blastula, the splanchnoderm forming at least a portion of the thinner median wall. In the anterior units, where the blastula retains its connection with the lateral mesoderm, this is undoubtedly the case.

Later Development of the Mesonephric Units.

Turning to the later development of the mesonephric unit, I have figured four stages (Plate 2, Figs. 20–23), all from the posterior half of the sixteenth somite of a larva 21 mm. in length (see Explanation of Plates). The first change in such blastulae as are shown in Figure 19 is the shortening of each to form a more nearly spherical mass (Fig. 20), which thus becomes larger in cross-section and more widely separated from its neighbors. Then there is formed a process which grows ventrad to touch, and later to fuse with, the peritoneum (Fig. 21, *nph'stm.*). In those anterior somites in which contact between blastula and peritoneum is present from the beginning, the same appearance is produced by a slight dorsal migration of the blastula, leaving a cone of cells joining it with the peritoneum. The thinness of the median wall of the blastula (Fig. 21) has become more marked than in previous stages. As above stated, this thinner median wall probably represents the splanchnic layer of the mesomer. The thickened lateral wall (presumably all somatic) sends out a conical proliferation of cells, which bends ventrad and presses its point against the dorsal wall of the duct (Fig. 22).

In the stage represented by Figure 23 the inner tubule has fused with the duct, and the dorso-median portion of the blastular wall has bent inward (*tbl. ms'nph.* and *cps. Bow. i.*). The mesonephric unit now presents in cross-sections of the body the form of a sigmoid curve. The incurved portion, just mentioned, has sometimes been described as forming the covering of the glomerulus. In Amblystoma, at least, it is only the median portion of the wall of the invagination (*cps. Bow. i.*) which forms the glomerular covering, the lateral wall (*tbl. ms'nph.*) allying itself entirely with the inner tubule.

The further development of the Malpighian body is as follows: the median wall of the infolding (*cps. Bow. i.*, Fig. 23), which is conspicuous as the most darkly staining portion of the entire unit, grows dorsad, stretching the tissue of Bowman's capsule (*cps. Bow.ex.*) to a very thin membrane. It then becomes curved to form a hollow hemisphere with the convexity directed toward the capsule, thus reducing the lumen of the latter to a narrow slit. The cavity of the hemisphere then becomes filled with cells, which increase in number until the whole forms a compact, darkly staining mass, the glomerulus. I am not certain as to the origin of the cells which fill the glomerulus. They may arise *in situ*, but I am inclined to think they are derived from small masses of cells which lie beneath the aorta, as there is often seen a connecting cord of cells between these masses and the glomerulus.

In Figure 23 the curves of the inner tubule all lie approximately in one plane. With increase in length, its curves become more complex and are no longer confined to the transverse plane. Figures 34 and 35 (Plate **3**), drawn from a wax reconstruction, show a stage in which the curves are still rather simple. The mesonephric unit in this case is from the eleventh somite of a larva 16 mm. in length and is almost functional (see Explanation of Plates). A comparison of Figure 34 with Figure 23 will show that the cone of cells (*nph'stm.*) connecting the unit with the peritoneum becomes the outer tubule. This later elongates somewhat.

At the point *crv. cp. Mpg.* (Fig. 23) the outer and inner tubules communicate with the lumen of the Malpighian body. In forms more primitive than the Amphibia, this condition persists. In Myxine (Maas, '**97**) the outer tubule may even open into the cavity of the Malpighian body at a point *opposite* the opening of the inner tubule. In Amblystoma, however, as in Ichthyophis (Semon, '**92**), the outer tubule *apparently* loses its direct connection with the Malpighian body and opens into the inner tubule at a short distance from the opening of the latter into the Malpighian body (Plate **3**, Fig. 24, *crv. cp. Mpg.*). This is brought about by a constriction of the Malpighian lumen just mesal to the point marked *crv. cp. Mpg.* in Figure 23, combined with a lengthening of the constricted region. The short, ciliated tube thus formed, which I shall call the *neck of the Malpighian body*, has generally been considered a part of the inner tubule. Viewed from the standpoint of its embryonic development, I do not see how it can rightly be so considered. Functionally, of course, it serves as an inner funnel, but, if my view of the extent of the two mesomeric layers is correct, it contains tissue from the splanchnic layer, — which no part of a true inner tubule does.

The differentiation of the blastula to form a complete mesonephric unit, as outlined above, will be found by comparison to agree quite closely with the similar processes in Pristiurus as set forth on page 49.

The further changes which take place in the mesonephric unit to fit it for functioning have been too well described for Urodela to necessitate a redescription. These changes consist in a widening of the various lumina, a great increase in the length and tortuousness of the inner tubule combined with a differentiation of its parts, and the establishment of a blood supply to the glomerulus.

Origin of the Dorsal Sets of Units.

In larvae about 20 mm. in length, the blastulae which develop into the primary units have become distinct as far back as the region of the opening of the duct into the cloaca. Soon after these blastulae have become well defined they are seen to be accompanied by smaller ones (*fnd. ms'nph. 2*, Fig. 26, Plate **3**), which are found from about the anterior end of the eighteenth somite caudad. These smaller blastulae are the *fundaments of the secondary units.* They are sometimes slightly removed from the primary blastulae, but generally in close contact with them. In the former case they may lie mesad to the primary ones, in the latter case they are always dorsad to them. *There is a single secondary blastula to each primary one.* Although these secondary blastulae may possibly develop from residual portions of the mesonephric blastema, they have every appearance of arising by a division of the primary blastulae.[1]

Cephalad, the secondary blastulae are generally considerably smaller than the primary, whereas caudad they may nearly equal the latter in size. This fact is apt to give the impression that the mode of division of the primary blastulae to give rise to the secondary ones (assuming that the secondary do so arise) alters as one passes caudad, but I think the true explanation is, that by the time the more posterior blastulae have divided, the growth of the *primary* units farther forward has outstripped that of their secondary companions (Fig. 25, *fnd. ms'nph. 2*), — since the latter remain almost unaltered for a considerable period during the metamorphosis of the primary units.[1]

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[1] As will be shown later, there is no possible doubt as to the origin of the secondary units from the primary ones in Rana sylvatica.

All that has been said in regard to the origin of the secondary units applies to that of the tertiary, with the exception that the latter arise by a splitting of, or proliferation from, the secondary blastulae, instead of from the primary. In Figure 27 one of these tertiary blastulae is represented in the process of splitting off from a secondary one. The section figured is especially significant from the fact that a dividing cell is seen between the secondary and tertiary blastulae.

The tertiary blastula, in its turn, remains unaltered for some time and then gives rise to a fourth blastula. As one passes caudad, the first tertiary fundament appears about half a somite behind the first secondary; the first quaternary a short distance behind the first tertiary, and so on (see Diagram 1, page 56, column *S*).

Later Development of the Dorsal Sets of Units.

Figure 25 is drawn from a section anterior to the region of the tertiary units. The primary unit presents the form of a sigmoid curve. The secondary blastula (*fnd. ms'nph. 2*) always lies against this curve at the upper limit of its lateral arm, which I shall call the collecting trunk (*trn. clg.*). The secondary blastulae which were described as lying dorsal to, and in contact with, the primary blastula have evidently simply retained that position, whereas those which were median to the primary blastula must have moved upward and outward to their present position. This change of position might be brought about by the growth of the Malpighian body of the primary unit, which would tend to force the secondary blastula upward and outward. In the development of the primary unit, the collecting trunk increases in length without becoming coiled, — the great mass of the unit being formed by the lengthening and coiling of the median arm of the curve (*tbl. ms'nph.*, Fig. 25). This fact allows the secondary blastula to retain undisturbed its position against the collecting trunk at the periphery of the mass.

With the increasing complexity of the primary unit, its Malpighian body and outer funnel, as well as the Wolffian duct, are shoved ventrolaterad until they lie at some distance from the mesentery (Fig. 24).

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[1] The early appearance of the secondary fundaments was a surprise to me, as all previous authors have stated that they are first visible when the primary units are far along in their development.

The Malpighian body is squeezed against the peritoneum, and the outer funnel opens slightly posterior and generally mesad to it.[1]

Nephrostomes of Secondary Units.

Soon after the primary unit has become functional, the secondary begins to go through the same process of development. Its behavior differs from that of the primary in two respects, however: its lateral arm empties into the collecting trunk of the primary unit instead of into the Wolffian duct; its outer tubule is not formed until the rest of its development is nearly completed. The development of this outer tubule is very difficult to decipher from the fact that, at the period when it begins, the Malpighian body has become imbedded in a mass of primary-tubule coils. I think, however, that I am not mistaken in saying that a hollow evagination, tipped with a solid, conical, deeply staining point, grows out from the neck of the Malpighian body, and, pushing its way through the mass of tubules, opens on the peritoneum mesad to the primary Malpighian body (Fig. 24, *nph'stm. 2*). This process begins in larvae about 45 mm. in length.

The later, more dorsal sets of units develop precisely as do the secondary. Each opens into the collecting trunk of the next ventral, so that there is formed a long *compound collecting trunk* reaching from the most dorsal set down to the duct.

Whether or not any or all of the units dorsal to the secondary one produce outer tubules, I do not know. In my oldest specimen[2] (from which Figure 24 was drawn), which had lost its gills, having left the water and assumed the adult markings, there are occasionally three outer funnels cut in one cross-section. In these cases the two younger nephrostomes are closely approximated to each other. This would seem to suggest that some of the tertiary units had developed outer tubules. The only way to settle this point with absolute certainty would be to follow each unit from its outer funnel to the common collecting trunk. This is practically impossible. A less certain but fairly satisfactory way would be to plot the outer funnels and all those glomeruli which, from their position, seem to belong to primary and secondary units. By comparing the number of outer funnels thus obtained with the sum of primary and secondary units, an inference could be drawn as to whether these two sets alone possess outer tubules or whether some of the tertiary also possess them. This I did throughout two somites in my oldest specimen with the following results: The number of outer funnels was somewhat *less* than the sum of the primary and secondary glomeruli, which points to the conclusion that only primary and secondary units possess outer funnels at this stage, — and possibly the same is true in the adult.[1] The fact that the sum of the primary and secondary glomeruli exceeds the number of outer funnels does not necessarily mean that some lacked outer tubules, but rather that some tertiary glomeruli were included in the enumeration. In fact, before comparing the numbers, I had marked some as possibly belonging to the tertiary set. The position of the glomeruli of the different sets is so variable at this stage that I was compelled to plot the openings of the collecting trunks into the duct in order to assure myself that what I took to be secondary glomeruli were not merely primary ones which had shifted their position dorsad. The number of openings found differed by only two from the number of those which I had considered as primary.

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[1] The section figured passes tangentially through the posterior wall of Bowman's capsule (*glm. 1*), which touches the peritoneum in the next anterior section.

[2] I unfortunately have no record of the length of the specimen, but should estimate it at about 50 mm.

The plotting also brought out the fact that, at this late stage, there has been no change in the relative number of units in the different sets. There is still one secondary for each primary (in the region of the secondary), one tertiary for each secondary, etc. Six or seven sets are present. All are functional except the two dorsal sets, of which the most dorsal consists of small, spherical, darkly staining blastulae.

Description of Table 1 and Diagram 1.

I wish now to call attention to Diagram 1 and Table 1, pages 56, 57. In the diagram I have plotted for the right side of the body in twenty-one individuals the position of the mesonephric units, the extent of the germ-cell mass, and the position of the opening of the duct into the cloaca. In the earlier stages the position of the units was determined by the point of greatest diameter in the blastula; in the later ones the glomeruli were taken as representing the units. In the case of the secondary, tertiary, etc., units, only the most anterior one is indicated. In Table 1 (page 57) I have translated the plotted units into numbers-per-somite for more ready comparison. By examining the diagram and table, the following questions can be answered: —

(1) Do any units degenerate and disappear?

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[1] Fürbringer ('78) states that in Salamandra maculosa the secondary units send outer tubules to the peritoneum. Hoffmann ('86), on the other hand, states that in Triton they do not.

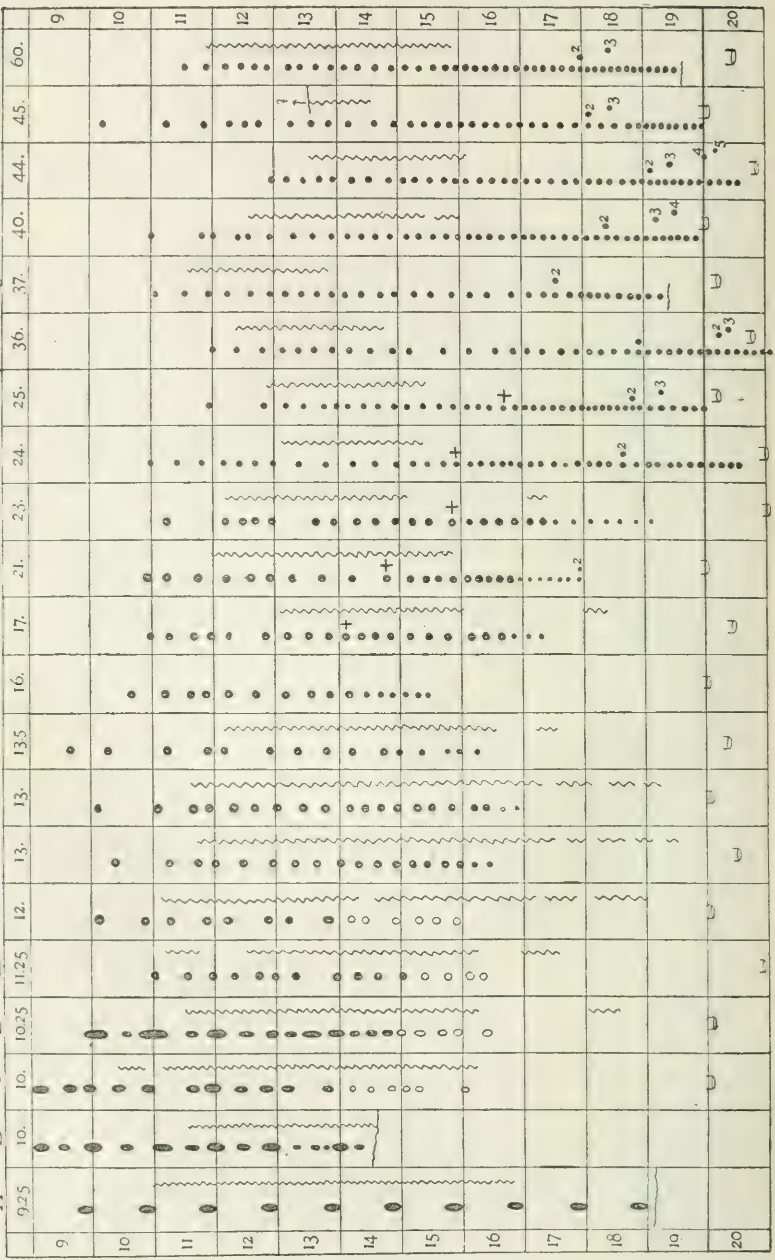

Diagram 1. (For explanation see opposite page.)

Explanation of Diagram 1.

Mesonephric units of Amblystoma plotted according to position in the somite. Individual larvae denoted by the letters *A* to *U*, with their lengths in millimetres immediately below. Somites 9 to 20 are numbered in the extreme right-hand and left-hand columns. The units are represented by solid circles or ovals, — *rings* indicating indistinct fundaments, whose number is uncertain. Circles numbered 2, 3, 4, and 5 (*L*–*U*) represent the most anterior of the secondary, tertiary, etc. units. The *wavy lines* indicate the extent of the germ-cell mass. *Crosses* (in individuals *K*–*O*) show the position of the most posterior of the *functional* units. The position of the opening of the Wolffian duct into the cloaca is shown at the bottom of the diagram (somite 20).

	B	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*	*N*	*O*	*P*	*Q*	*R*	*S*	*T*	*U*	*B*–*K*	*L*–*U*	*B*–*U*	Probable Number of Units.
9	2	2						1													.5	0		1
10	2	2	2		2	1	2	1	1	1	1		1						1		1.4	.3	Average of all.	1 to 2
11	2	2	2	2	2	2	2	2	3	3	2	1	2	1	1	3	1		2	2	2.2	1.5		2
12	2	2	2	3	2	3	3	2	2	2	3	4	3	1	2	3	2	1	3	4	2.3	2.6		2
13	3	2	3	2	2	3	2	2	3	3	2	2	3	4	3	4	3	3	3	4	2.4	3.1		2 to 3
14			4	3	3	3	4	3	4	4	2	3	3	4	4	4	3	4	3	4	3.5	3.4		3 to 4
15					3	4	3	3		3	3	3	4	4	2	3	4	5	5	5	Average of younger ten.	Average of older ten.	3.6	3 to 4
16											6	4	6	5	3	3	5	5	5	6			4.7	4 to 5
17											6	4	5	7	4	5	5	5	4	7			5.2	5
18													5	8	5	6	6	5	5	8			6.0	6
19													6	6	6		6	7	8				6.5	
20													4		7		7	5						

Table 1.

Showing the number of units to each somite. The individual larvae are designated by the letters *B* to *U*; the somites, by the numbers (9 to 20) in the first column.

(2) Are any primary units added in any somite after the first unit in that somite has appeared?

(3) What is the typical number of primary units for each somite?

It will be seen that in somites 9, 10, and 11 there are more units in the younger than in the older larvae. In attempting to account for this fact, two possibilities must be considered; either some of the units degenerate or they migrate caudad, thus shortening the mesonephros. In order to determine which of these alternatives is the true one, a line was drawn (Table 1) separating the ten larvae 17 mm. or less in length (*B–K*) from the ten which were over 17 mm. in length (*L–U*),[1] and the average number of units in each of the six anterior somites on one side of the line (*B–K*) compared with the average of the corresponding somites on the other side (*L–U*). The averages for somites 9, 10, and 11 of the younger ten (0.5, 1.4, 2.2) are *greater* than those of the older ten (0, 0.3, 1.5). The averages of somites 12, 13, and 14, on the contrary, are in general *less* for the younger than for the older ten (2.3, 2.4, 3.5, as compared with 2.6, 3.1, 3.4). The decrease in the number of units in the anterior somites as the larva grows older is thus probably due entirely to a shortening of the mesonephros. The preponderance of the units in the older ten, in somites 12, 13, and 14, over those in the younger ten, does not, however, quite make up for the paucity in somites 9, 10, and 11 of the older ten, and the conclusion is natural that the shortening of the mesonephros has also affected the region of somites 12, 13, and 14, and that on investigation it would be found that there had been a backward movement of units from this region into the somites immediately behind. Pursuing the method employed above, I divided the ten older larvae into two lots. On counting the units in somites 15, 16, and 17 of each lot, I found the sum of the units in the younger five to be sixty-six, that in the older five, seventy-two; that is, there is a preponderance of 0.4 of a unit per somite, for somites 15, 16, and 17 in the *older* five over those in the *younger*. As this preponderance is not large enough to suggest an actual addition of new units in each somite, it is confirmatory proof of a shortening of the mesonephros.

In answering question 1, question 2 has also been answered in the negative, at least for all but the one or two most posterior somites. There remains the question: What is the typical number of units for each somite? The simplest way to determine this would seem to be to find the average for each somite in the twenty larvae. As the arrangement of the units has been much modified in the more anterior somites of the older larvae, I think a more significant series of averages is found by using for the six anterior somites only the ten younger larvae (columns *B–K*).

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[1] Larva *A* of the diagram is not included in the table.

I should not include the average for somite 19 (6.5), as I do not think it trustworthy, from the fact that the mesonephros curves ventrad in that somite and hence is cut more or less frontally, which renders the determination of the number of units difficult. It is this curving which sometimes causes the units to appear as if they extended posterior to the opening of the duct into the cloaca, as in larva *P.* of the diagram.

In discussing the significance of these averages, I will first call attention to the diagram of larva *A*, which shows a single fundament for each somite, from the ninth to the eighteenth at least. It will be remembered that, while in posterior somites these fundaments could not be identified in the continuous blastema formed from the fused mesomers, in anterior somites they remained distinct and became the "blastulae of the first order," between which there soon appeared the smaller "blastulae of the second order." In the anterior four or five somites, usually not more than one of these smaller blastulae appeared between each two of the first order. Caudad, they appeared in increasing numbers. From the last column of the table (p. 57) it seems that the blastula of the second order is generally absent from somite 9, sometimes present in somite 10, and typically present in somites 11–13. In somite 13 a second one is sometimes added, making the total number of blastulae two to three. This process continues, an extra blastula being generally added in every second somite, as represented in the last column of the table (p. 57).

Primary and Secondary Units in Amblystoma and Ichthyophis.

It will be instructive to compare the condition in Amblystoma with that found in Ichthyophis by Semon ('**92**). In Ichthyophis the tubules of the first set are strictly segmental in position and resemble in structure the primary units in Amblystoma. Soon there appears a second set; these are first seen as small intersegmental balls of cells (blastulae), resting on short outgrowths from the duct. Then appear in succession a third, a fourth, and a fifth set, all in line (horizontally) with those of the first set, and *all opening into the duct.* Semon suggests that we have here a hint as to the origin of the dysmetamerism found in the higher Amphibia and in the Amniota, where, he suggests, the multiplication of the fundaments may take place very early. He assumes that in Ichthyophis these second units arise by a budding from those of the first set, for he finds them connected with the Malpighian capsule of the latter by a cord of cells which soon disappears. He further suggests that a similar division of a [theoretical] first set takes place in the higher Amphibia. From my description of the formation of the blastema (see Stage V) it would seem that Semon's surmise as to the origin of the dysmetameric arrangement of the units in the higher Amphibia contains the essence of the truth. A more exact comparison between the process in Ichthyophis and that in Amblystoma seems to me to be as follows: in Ichthyophis that portion of the mesomer of each somite which remains after the detachment of the sclerotome becomes *entirely* converted into a single, segmental, mesonephric blastula. Any later units must therefore be derived *from this first one* by splitting or budding. In Amblystoma, the division into several units per somite takes place so early that the *mesomer itself* is directly differentiated into the full complement of primary blastulae belonging to its somite. Of these primary units, one (that of the "first order") in each somite appears earlier than the rest and is probably homologous with the "primary" units of Ichthyophis. The later primary units (those of the "second order") in Amblystoma, which soon appear between the earlier ones, are probably homologous with the second, third, etc., sets of Ichthyophis.

In Ichthyophis, the development of the second, third, etc., sets of units is followed, after a long period, by the appearance of two *dorsal sets*, whose origin is unknown. There is some evidence that these open into the tubules of the earlier units, but that point was left by Semon unsettled. It seems probable that these "dorsal sets" of units in Ichthyophis are alone strictly homologous with the secondary, tertiary, etc., sets of Amblystoma.

There is little doubt in my mind that the second, third, etc., sets of units in Ichthyophis represent a stage in phylogenetic development between the typical secondary (dorsal) units and the extra primary ones (those of the second order) of Amblystoma. During this evolution, the buds from which they arise have shifted their point of origin from the *dorsal* side of the primary blastula to its anterior (or posterior) side. This fact explains their connection with the duct instead of with the collecting trunks of the primary units. The new mode of opening has the great advantage of making them wholly independent of the primary units, so that they are free to mature as early as the primary. The theory set forth above offers an explanation of the fact that these second, third, etc., sets in Ichthyophis occupy a curious intermediate position between the primary and the true secondary (dorsal) units of Ichthyophis and Amblystoma. They resemble the primary in their position and in the fact that they open into the duct; they resemble the true secondary units in the fact that their outer tubules are formed after the unit has become quite complex, and in the fact that they do not form connections with the testis. Semon himself suggests that in Urodela the connecting of *all* of the primary units with the testis is a secondary acquirement.

Relations between Sexual and Secretory Portions of the Mesonephros.

As is well known, the mesonephros in Urodela is divided into a sexual and a secretory portion. The former is connected with the sexual glands by outgrowths from Bowman's capsules, permanently in the male, temporarily in the female. In the male the outer funnels of this portion of the kidney close and disappear. The secretory part, which lies posterior to the sexual gland and does not form connections with it, is characterized by the presence of dorsal sets of units. I will next consider the extent of these two parts in Amblystoma. From Diagram 1 it will be seen that scattered germ-cell masses may extend as far back as somite 19 (larva *G*). In the older larvae (from 23 mm. in length), however, the germ cells become restricted without exception to somites anterior to the sixteenth. Thus the sexual part of the mesonephros cannot extend back of the anterior end of somite 16. The rest of the kidney is to be considered as belonging to the secretory part. It will be noticed that a criterion which is generally used in defining the secretory part, namely, the presence of dorsal units, does not apply to the whole of the part, for those units do not extend farther cephalad than the posterior end of somite 17. Although they *may* be added to somites 17 and 16 at a later date, it seems very improbable that they are, from the fact that the formation and development of those already present progressed from in front backward. The posterior portion of the secretory part is thus distinguished from the anterior portion by an anatomical character, — the presence of dorsal units. Significance is given to this difference in anatomical character by a peculiarity in the development of this region of the mesonephros which, although not conspicuous, is to be considered as of some importance from a phylogenetic standpoint. The peculiarity consists in a *retardation in the appearance and development of the blastulae.* When the blastulae first become distinct, they extend back to the region of somite 16 (see Diagram 1, larvae *C*, *D*, etc.). From that stage to one represented by larva *J*, — a period of rapid development during which the animals have added six millimetres to their length, — there is no further caudad extension of blastulae, the developmental processes being restricted to a maturation of the units already formed. In larvae about 16 mm. in length, this maturation process has resulted in the conversion of the three or four most anterior blastulae into functional units. As soon as this occurs, the addition of blastulae is resumed and progresses steadily through somites 17, 18, etc., so that in a larva of 24 mm. (*N*) all or nearly all of the primary units have appeared. During this process, this delayed set of primary tubules seems to remain quiescent until all have appeared. This fact explains the *sudden* transition, in the region of the sixteenth or seventeenth somite (larvae *K*, *L*, *M*), from complex units to simple blastulae as one follows the organ caudad.

Since Semper ('**75**) first suggested it, various authors have seen in the secretory portion of the Amphibian mesonephros a forerunner of the metanephros of the Amniota. Felix ('**97**) believes that it contains both mesonephric and metanephric elements, and Nussbaum ('**97**) takes a similar view. It seems to me that the phenomenon just recorded — the *retarded development* of a part of the mesonephros — is important evidence in favor of the view that a portion of the secretory part (that posterior to somite 16) is comparable with the metanephros of higher forms, for one of the chief peculiarities of an excretory system which is divisible into meso- and meta-nephros is a chronological break in its development.

B. RANA.

The earlier processes in the development of the somites in Rana are very similar to those in Amblystoma. The chief difference lies in the fact that development is more hurried and the cavity of the epicoelom becomes sooner filled by the ingrowth of the muscular tissue developed from the splanchnic wall. The sclerotome seems to arise essentially as in Amblystoma, from the splanchnoderm of the ventral wall of the somite. I apply to Rana the same conception as to the extent of the mesomer as in the case of Amblystoma.

The first figure (Fig. 6, Plate **1**) is of a section through the middle of the sixth somite [1] of a larva 3.25 mm. in length, which corresponds to the stage of Amblystoma illustrated by Figure 13, Plate **2**. Although the three pronephric nephrostomes are open, the Wolffian duct has not yet reached the cloaca. About fifteen somites are recognizable. No blood-cells have as yet appeared.

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[1] In numbering the somites, the three pronephric nephrostomes are considered as opening in the second, third, and fourth somites.

As shown in the figure, splanchnoderm (*spl'drm. l.*) and somatoderm (*so'drm. l.*) are pressed together, so that the arrangement of the cells and the presence of deeper pigmentation alone indicate the line of demarcation. Both layers are seen to be continuous throughout the three regions of the mesoderm, — epimer, mesomer, and lateral plates. Toward the posterior end of each mesomer the coelom is represented by an actual lumen.

Figure 14 (Plate 2) represents a section through the sixth somite of a larva 4.5 mm. in length and corresponding approximately to the stage in Amblystoma illustrated by Figure 2. The Wolffian ducts are just opening into the cloaca, and blood-cells are numerous in the ventral and lateral lacunae of the entoderm. Occasionally one is seen to have migrated dorsad into the fundament of the blood-vessels (*va. sng.*). In the figure, the sclerotome is seen as a proliferation of the mesomeric splanchnoderm. In sections posterior to the one figured, it extends to the dorsal angle of the epimer. In the outer angle of the epimer is seen a small lumen, caused by the separation of the somatic and splanchnic layers. Later, this lumen is obliterated, as in Amblystoma, by a disintegration of its lateral wall (compare Fig. 4, Plate 1). The mesomer (*ms'mer.*), leaving out of account the sclerotomic portion, is less clearly marked than in Amblystoma, but careful study shows that it contains tissue belonging to both somatic and splanchnic layers.

In the larva represented by Figure 4, the obliquity of the somites has increased to such an extent that a section through the centre of one cuts the next anterior one as well. The lower somite in this figure is the ninth, the upper one the tenth. The larva measures seven millimetres and shows a well-developed glomus and ciliated nephrostome.

That Figures 6 and 14 represent sections anterior to the position of the future mesonephros is immaterial, as there is no essential difference *at those stages* between the mesonephric region and that immediately in front of it. However, in the larva now under discussion and in older ones, sections through the mesonephric region and those through the region anterior to it present very different aspects, for, while anteriorly the mesomer remains inconspicuous, in the mesonephric region it becomes more and more massive, as may be seen from Figures 4, 16, and 15, *fnd. ms'nph.* The statement that the *mesomer* becomes massive is not quite accurate. What really happens is that the mesomers of successive somites fuse to form a flattened band of tissue, the *mesonephric blastema*, in which local swellings appear. The section represented by Figure 4 passes through such a swelling. It will be seen (even more clearly in Figure 16) that, on the one hand, the mesomer is now entirely severed from the sclerotome, and on the other retains only an indefinite connection with the somatic layer of the epimer.

In the particular larva from which Figure 4 is taken there is one swelling in the blastema for each of the somites 8, 9, 10, and 11 (see larva *B*, Diag. 2, p. 65). This would lead one to suppose that each swelling corresponds to one of the original mesomers. Such a supposition, if true in this particular case, cannot be so generally, as there are usually more swellings than somites. On plotting the swellings in thirteen larvae (*A–M.* in Diagram 2) varying from 6.5 mm. to 9.5 mm. in length, it was found that

somite 7 contains an average of 0.08 swellings
8 " " 1.08 "
9 " " 1.23 "
10 " " 1.6 "
11 " " 1.8 "
12 " " 0.4 "

In other words, the seventh somite occasionally develops a swelling; somite 8 has generally one, rarely two; somite 9 often has two; somites 10 and 11 have more often two than one, and somite 12 shows an occasional one. As these numbers agree fairly well with the number of primary units in the fully developed mesonephros, the conclusion suggests itself that the swellings are the fundaments of the blastulae. On that assumption, it is true, there are not enough swellings in the posterior somites, especially somites 11 and 12; but it is clear that in some cases they have not all appeared, and it is also possible that some of the swellings contain the fundaments of more than one blastula. As development proceeds, their appearance confirms the idea that they are young blastulae. They increase in diameter, and the deeply staining nuclei show a radial arrangement (see Fig. 15, Plate 2). These blastulae are spindle-shaped and more elongated antero-posteriorly than in Amblystoma, and are usually, although not always, continuous with each other by their tapering ends. There are thus formed cord-like connections, which are retained in many cases to a late period; these connecting parts may even become massive, and seem to contribute a portion of the tissue from which the secondary units are formed.

It will be recalled that in Amblystoma the fundaments of the " units

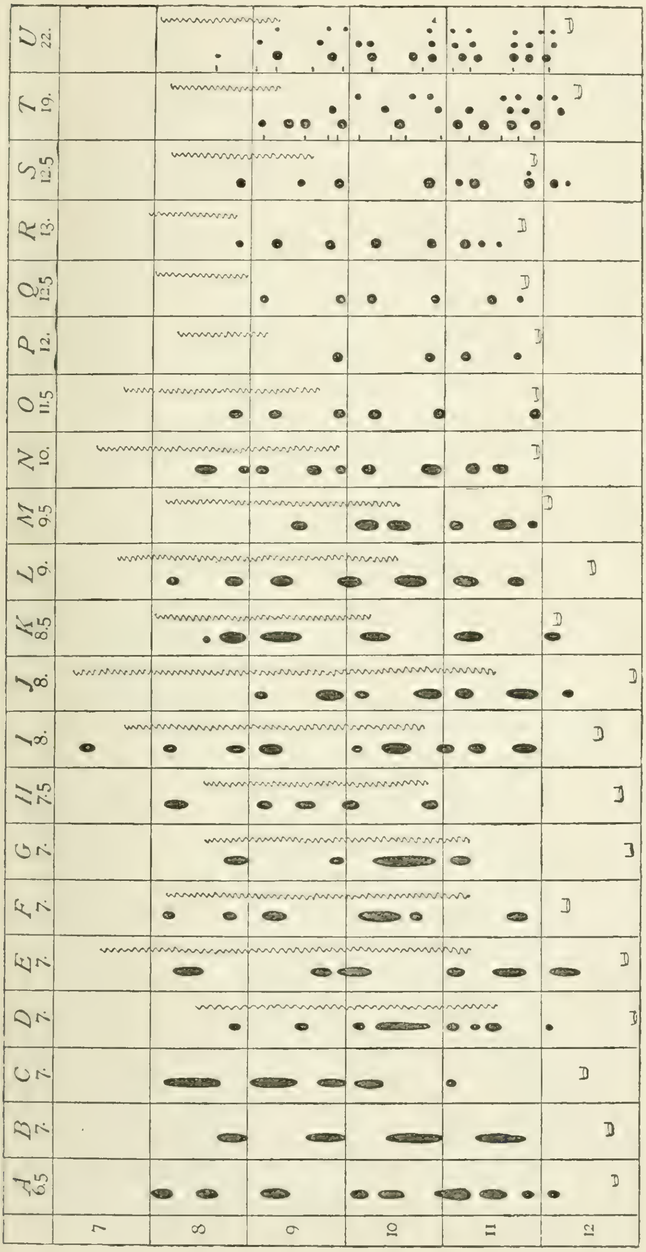

DIAGRAM 2.

Mesonephric units of Rana. For explanation of signs see Explanation of Diagram 1 (p. 57). In larvae *T* and *U*, primary, secondary, and tertiary units are plotted in separate vertical columns, and the openings of the collecting trunks are indicated by a short horizontal line.

of the first order," one for each somite, appeared in the posterior part of the mesomers before these were cut off from the epimers to form the mesonephric blastema. The question naturally arises as to whether there is in Rana any such sign of the primitive metameric arrangement of the mesonephric units. I find no such condition as that described in Amblystoma. The only hint at a segmental arrangement is found in a few cases (Diagram 2, larvae *B*, *G*, *K*, *P*), where there is but one "swelling" per somite. As suggested above, some of these swellings may really contain the fundaments of more than one blastula. This is difficult to believe, however, in such cases as larvae *K* and *P*, for there the swellings are in the shape of distinct blastulae (Figure 15 shows the one in the twelfth somite of larva *K*), and it seems probable that, were any of them going to divide, some signs of the fact would already be observable. It seems possible, then, that such cases show an atavistic condition, and that if a large number of *adults* were examined, cases might be found showing the primitive condition of a strictly segmental arrangement of the *primary* units of the mesonephros.

Development of Primary, and Origin of Secondary, Blastulae.

As development proceeds, the blastulae grow more massive and resemble those of Amblystoma, with the exception already mentioned that their anterior and posterior ends are prolonged to form a connecting cord. In later stages, this cord is often clearly interrupted, generally nearer the posterior than the anterior blastula. Even when the cord seems to be continuous, it is possible that there is really only close *contact* between successive blastulae. Figure 102 (Plate **8**) shows the relative size of two blastulae and connecting cord in the posterior portion of the mesonephros, — where the blastulae, being more numerous, are naturally more closely approximated than farther forward. This figure was made by superposing drawings of six consecutive frontal sections.[1] One of the sections used in the reconstruction is shown in Figure 18, Plate **2**. As the blastula was cut obliquely, only its posterior prolongation shows.

For the sake of clearness and brevity I have illustrated the differentiation in a mesonephric unit by a series of six diagrammatic cross-sections (Figs. 94–100, Plate **8**). Figure 94 represents a cross-section at the stage when the blastula has the form of a simple, longitudinally elongated spindle. The next step in the process, represented in Figure 95, is the partial division of the blastula into a smaller dorsal and a larger ventral chamber. In this partial division, the anterior and posterior prolongations become associated exclusively with the dorsal chamber, which thus takes on the form of a longitudinal tube whose ends are, at least generally, in close contact with the similar tubes (that is, upper chambers) of the adjacent units. This condition will be made clear by a glance at Figure 30 (Plate **3**), which is a dorso-median view of a reconstruction in wax of four blastulae and the Wolffian duct from the right side of a larva. The two anterior blastulae are approximately at the stage represented in Figure 94, the third in that represented in Figure 95. The ventral chamber is destined to form the *primary mesonephric unit.* The dorsal chamber remains without change for a considerable period and gives rise successively to the *dorsal sets* (secondary, tertiary, etc.)

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[1] This method gives only a silhouette and hence affords no proof of the continuity of the connecting rod.

The primary blastula (lower chamber) enlarges, its median and lateral walls showing a decided difference in thickness. The lateral wall, which is the thicker, buds out an evagination, which abuts on the duct. This stage is represented in Figure 96 (Plate **8**), and in the most posterior unit of the wax model (Fig. 30, Plate **3**). A section corresponding in position to the dotted line of Figure 30 is shown in Figure 28.

At the next stage (Fig. 97, Plate **8**) we recognize that the evagination in Figure 28 is the fundament of the inner tubule. It has grown in length, its free end (*tbl. ms'nph.*) has been crowded upward, and there it becomes attached to the duct. Ventro-median to the fundament of the inner tubule, the wall of the blastula again evaginates and proliferates cells to form a cone extending toward the peritoneum (Fig. 97, *tbl. nph'stm.*). This is, of course, the young outer tubule. At a stage as early as that of Figure 97 the fundaments of all the parts of the complete mesonephric unit can be recognized; the inner tubule, the outer tubule, and the glomerulus (exclusive of the vascular part), the last consisting of the glomerular covering (*cps. Bow. i.*) and Bowman's capsule (*cps. Bow. ex.*). Having determined the positions of the various fundaments in the present stage, we can now identify their positions in the preceding stage (Fig. 28), if we bear in mind that between these two stages the unit has rotated some forty degrees about its longitudinal axis (compare Figs. 96 and 97). In Figure 28 the fundament of the outer tubule (*tbl. nph'stm.*) composes the ventral wall of the blastula median to the evagination which forms the inner tubule (*tbl. ms'nph.*). The fundament of Bowman's capsule is contained in the tissue of the thin median wall (*cps. Bow. ex.*), and the glomerular covering is derived from the dorso-lateral portion of the thick lateral wall (*cps. Bow. i.*).

The succeeding diagrammatic figures (98, 99, and 100) scarcely need explanation. The only differences between the condition shown in Figure 99 and that seen in actual sections are: (1) the outer tubule is not encountered in the same section as the inner tubule, because it enters the Malpighian neck from a slightly anterior direction; and (2) the fundament of the dorsal sets of tubules (*fnd. ms'nph. 2*) is also displaced, being generally posterior, though sometimes anterior, to the rest of the unit, and hence is not seen in a section through the centre of the blastula. Although on account of this displacement the relationship between the dorsal fundament and its parent cannot be shown in a single section in the case of the primary unit, it can be shown in the case of the secondary, where often there is no displacement. Thus Figure 29 (Plate 3) shows the secondary blastula and the fundament of the more dorsal sets (*fnd. ms'nph. 3*), connected by an attenuated cord. A corresponding stage of the primary unit is represented in Figure 100.

To recapitulate briefly, the fundament of the dorsal sets of units is developed from a "dorsal chamber" constricted off from the primary blastula. This dorsal chamber, at first tubular, becomes a typical blastula, probably by a shrinking or shortening of the tube to form a spherical mass. With the growth of the mesonephric mass, the dorsal fundament is forced farther from the parent one and is finally severed from it. For some time, however, the two remain connected by a cord of cells, which indicates clearly the point of origin of the dorsal fundament, — the dorso-median region of Bowman's capsule.

In comparing the history of the fundaments of the dorsal sets of tubules in Rana with that in Amblystoma, we see that the only essential difference is that in Rana each fundament remains connected for a considerable period with the primary unit from which it took its origin, instead of being entirely cut off from it at an early period, as in Amblystoma.[1]

The development of the tertiary from the secondary, the quaternary from the tertiary, etc., shows the same difference in the two animals.

In the later development of the dorsal units, the opening of each into the inner tubule of the next ventral (parent) one, is established in a manner similar to that traced in Amblystoma, and as far as could be determined, there is one secondary for each primary (throughout the range of the secondary), one tertiary for each secondary, etc. The development of additional dorsal units is continued to a surprisingly late period. Thus I found young, non-functioning units in a large, adult female of Rana virescens.

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[1] The secondary units in the earliest stages seen by Semon ('92) in Ichthyophis consisted of spherical masses connected with Bowman's capsule by a cord of cells. From this he suggests that probably they had recently budded out from the Malpighian body.

Outer Tubules and Nephrostomes.

As is well known, the outer tubules in Anura, after reaching the peritoneum, become cut off from the rest of the unit and open into venous spaces (see Fig. 100). This fact I confirmed in Rana sylvatica, Rana virescens, and Hyla versicolor in sections of the larvae, where the cilia at the inner end of the tubule can be very plainly seen extending into venous spaces. In R. virescens I also confirmed it in the adult by means of the method — first used by Nussbaum ('80), I believe — of injecting powdered carmine into the body cavity of the living animal, then fixing and sectioning.

It seems still undecided whether, in Anura, the outer tubule affords at any stage an actual communication between the coelom and the neck of the Malpighian body. At the time when it is still joined to this neck and to the peritoneum it possesses a lumen throughout a part of its length, but more I cannot affirm. Whether the lumen is continuous with the coelom and with the cavity of the Malpighian neck, I found it impossible to determine, as cross-sections of the animal cut it at an unfavorable angle. After all, the continuity or discontinuity of the lumen is a matter of small theoretical importance, as it is perfectly clear that the outer tubule arises exactly as in the Urodela, and that the adult peculiarity is ontogenetically acquired. A matter of more importance is the origin of the large number of nephrostomes which appear as the animal matures. Are they (1) the openings of outer tubules which grow down independently and become cut off from the dorsal sets of units; are they (2) evaginations of the peritoneum; or are they (3) developed from the original outer tubules by division? The second and third suppositions seem both to be true. The outer tubules (in an old larva of R. virescens) have every appearance of dividing, and occasionally there are also to be seen short, deeply staining, peritoneal evaginations at a considerable distance from any older ones. It is of course possible that these arise from cells derived from dorsal units, but I have looked in vain for cell-strands growing out from those units toward the peritoneum.

Order of Appearance and Number of Primary Units.

It is usually stated that in Anura the posterior mesonephric units are the first to appear, the more anterior ones following in order. This is not strictly true for R. sylvatica. As will be seen from Diagram 2 *(A–T)*, from four to nine primary fundaments, in the shape of swellings in the blastema, appear *simultaneously*. Of these the more posterior *develop* most rapidly, so that in passing cephalad one finds the blastulae less and less mature.

In none of the larvae nineteen millimetres or less in length whose mesonephric units are plotted in Diagram 2 *(A–T)* are there more than nine primary units. In larva *T* there are twelve collecting trunks opening into the duct; in larva *U* there are eleven, and in one individual (32 mm. long), not represented in the diagram, there are *fifteen*. There are two possible explanations of the preponderance in the number of these collecting trunks over that of primary units in the younger specimens. Either new primary units have been added, or some belonging to the secondary set have sent their tubules directly to the Wolffian duct instead of to the collecting trunks of the primary units. That the number of tubules opening into the duct in larva *T* exceeds by three the number of units plotted as primary ones, seems to favor the latter view, but it must be borne in mind that the units were plotted as belonging to the primary or secondary set solely from the position of the Malpighian body, and that this may easily have been displaced by crowding. Whether or not secondary units have simulated primary ones by sending their tubules directly to the duct, there is no doubt that true primary units are added to the original series, for in both larvae *T* and *U*, young, deeply staining ones are seen at both the posterior and anterior ends of the kidney. Even in the older larva (32 mm. long) mentioned above, immature primary tubules are seen at the posterior end of the series. In this larva the most anterior tubule on either side of the body, although it is clearly functional, is not accompanied by any sign of a fundament of dorsal sets. From this it seems probable that at least one tubule may remain simple throughout life.

Diagram 2 (p. 65) shows that there is some shortening of the mesonephros. Much more striking is the shortening of the germ-cell mass (principally the posterior portion), which either shrinks by a rearrangement of cells or undergoes degeneration. The same phenomenon was noted in Amblystoma.

C. COMPARISON OF THE MESONEPHRIC FUNDAMENTS OF AMBLYSTOMA AND ICHTHYOPHIS.

As the most important of my conclusions relates to the derivation of the mesonephric fundaments, I have endeavored to make clear, by Figures *E–H* (p. 72), the similarity between the mode of formation of the fundaments in Ichthyophis and Amblystoma. The comparison of the two forms has importance not only from the fact that Ichthyophis is a primitive Amphibian, but mainly from the fact that it is comparatively easy to homologize the processes in the formation of the mesonephric fundaments in that animal with those in elasmobranchs, a group which has (in my opinion) a very primitive or generalized mesonephros.

Figures *E* and *F* are based on Semon's ('**92**) Figures 9 and 10, the interpretation of the extent of the various portions of the mesodermic layers being, however, entirely my own. According to Semon's description, the development of a mesonephric fundament is briefly as follows. In the earliest stage shown by him the cavity of the somite is already separated from the body cavity by the fusion of somatoderm and splanchnoderm (see Fig. *E*). The cavity of the somite then becomes divided into a larger upper and a smaller lower chamber by the development of a cross-partition (Fig. *F*), which thus forms the ventral wall of the upper chamber and the dorsal wall of the lower. The partition then splits into two layers, of which the dorsal becomes a part of the now solid epimer (the lumen having become filled by proliferation from the median wall), and the ventral furnishes a part of the wall of the lower chamber. This chamber is the mesonephric blastula. Its region of contact (Fig. *F*, *nph'stm.*) with the lateral mesoderm becomes broader; then by the ingrowth of connective tissue the contact is interrupted except at two points, a lateral (Semon's "Contact *a*") and a median ("Contact *b*") region.[1] The lateral region is destined to form the outer tubule and nephrostome; the inner the sexual cord.

The direction and character of the shading will make clear what portions I consider homologous in Figures *E*, *F*, *G*, and *H*.

Figure *G* is based on the stage of Amblystoma represented by Figure 13 (Plate **2**), Figure *H* on that represented by Figure 7 (Plate **1**).

During the process which in Ichthyophis (Fig. *F*) separates the mesomer ("lower chamber") from the epimer and converts it into a mesonephric blastula, its cavity remains as an actual lumen. In

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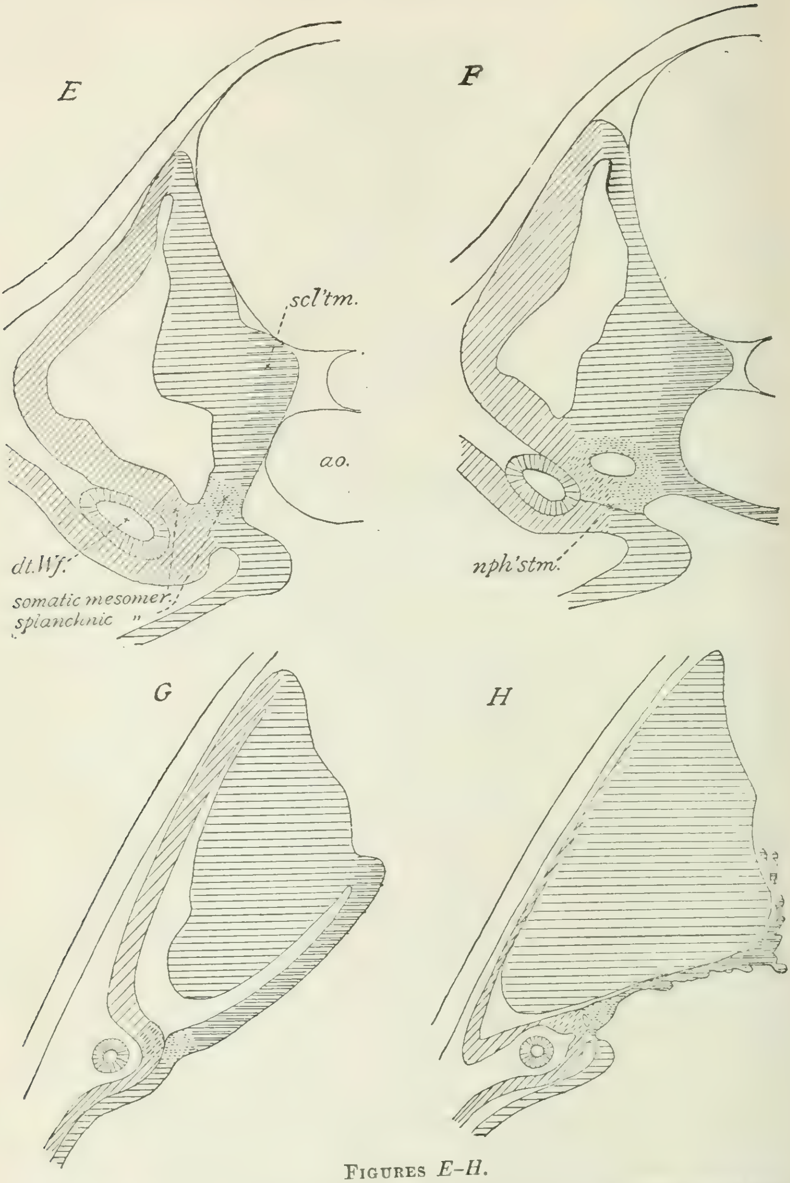
[1] For the sake of simplicity this later differentiation has been omitted in Figure *F*.



Figures *E–H*.

Four diagrammatic figures illustrating the similarity in origin of the fundament of a mesonephric blastula in Ichthyophis (*E* and *F*) and Amblystoma (*G* and *H*). The splanchnoderm of both epimer and lateral mesoderm is shaded with horizontal lines, that of the mesomer (exclusive of the sclerotome, which is indicated by double horizontal lines) with horizontal dashes. The somatoderm of both epimer and lateral mesoderm is shaded with oblique lines, that of the mesomer with oblique dots and dashes.

Amblystoma, on the contrary, the somatic and splanchnic layers of the mesomer are closely applied to each other during its severance from the epimer, and the lumen becomes apparent only in the well-formed blastula. I have consequently anticipated the conditions somewhat in giving the mesomer a lumen in Figure *H*. Aside from this the only important difference in the processes in the two animals is the formation of the nephrostome in Amblystoma by a secondary fusion of the outer tubule with the peritoneum. It seems probable, it is true, that some of the anterior nephrostomes in Amblystoma are formed as in Ichthyophis, from a persistent connection with the coelom, but posteriorly (and throughout the entire mesonephros in Rana) the connection is certainly a secondary one.

Although, as just stated, the final connection between mesomer and lateral plates (by means of the outer tubule) is a secondary one in Amblystoma and Rana, it seems probable that the two layers of the mesomer retain their relative positions and give rise to the same portions of the mesonephric unit as in Ichthyophis and elasmobranchs. What I believe these portions to be will be found illustrated in Figures 94–100, where the shading of the supposed somatoderm is made with continuous lines, that of the splanchnoderm with interrupted lines.

D. RECAPITULATION OF THE MESONEPHRIC DEVELOPMENT.

Amblystoma.

1. The mesonephric blastula is derived from a portion of the somite which is homologous with the mesomer of elasmobranchs, and it contains both splanchnoderm and somatoderm.

2. Contrary to the condition in elasmobranchs and Ichthyophis, the lumen of the mesomer at the time it is cut off from the epimer is only *potential;* it first appears as the cavity of the mesonephric blastula.

3. The more anterior blastulae probably never lose their connection with the two layers of the lateral mesoderm. The outer tubules (at least in the anterior primary units) therefore contain both somatoderm and splanchnoderm. In this connection it was noted that the morphologically dorsal angle of the body cavity is not at the upper limit of the mesentery, but lies just mesad to the Wolffian duct. Hence the germ cells (except in very early stages) all lie in the splanchnoderm.

4. The secondary character of the dysmetamerism of the urodele mesonephros shows itself in the fact that the primary blastulae can

be divided into two sets, in one of which the elements (those of the "first order") are metamerically arranged. It is suggested that the units of the "second order" represent the final product of a phylogenetic evolution in which a number of secondary units have been transformed into apparently primary ones. Their similarity to the true primary units (those of the first order) is due to the fact that they now arise almost simultaneously with these, and hence are developed under identical conditions. It is further suggested that the units of the second, third, etc., sets in Ichthyophis probably represent a stage in the evolutionary process intermediate between typical dorsal units and the extra primary ones (those of the second order) of Urodela, in that they connect with the duct, rather than with the tubules of the primary units, but have not as yet formed connections with the gonads.

5. One of the results of the early appearance of the extra primary units (those of the second order) in Urodela is that, instead of the entire mesomer of each somite being converted, as in Ichthyophis, into a single mesonephric blastula which gives rise to later generations by budding, the mesomer itself gives rise directly to more than one unit.

6. The appearance of the fundament of the dorsal sets of units takes place much earlier than has been hitherto supposed. They arise by a splitting or budding process from the primary blastulae shortly after the latter are formed, and while they are still small and simple. The tertiary sets arise in a similar manner from the secondary, the quaternary from the tertiary, etc.

7. At the period of the animal's metamorphosis only primary and secondary units have produced outer tubules, and in all probability the outer tubules are confined to these two sets of units throughout life.

8. By plotting the positions of the units at various ages it is learned that after the primary units are clearly differentiated in a somite no more are added, the apparent increase in number in certain somites being due to a concentration of the whole organ. There seems to be no degeneration of units in the ages examined.

9. The plotted diagram (p. 56) also brings out the fact that the appearance and development of units, caudad, is not uniform, there being a delay between their completion in the sexual and an anterior part of the secretory portion and their beginning in the remainder of the secretory portion. This fact is considered as important additional evidence in favor of the view, first suggested by Semper in 1875, that the secretory portion of the mesonephros in some of the Anamnia is the morphological representative of the amniote metanephros.

10. The extent of the germ-cell mass, caudad, becomes less as the animal grows older. This is due to a total disappearance of the germ cells, as such, they being transformed in all probability into ordinary peritoneal cells. A similar transformation is suggested to account for their disappearance from the somatoderm in the sexual portion of the mesonephros.

Rana.

1. As in Amblystoma, the mesomer contains tissue from both somatoderm and splanchnoderm. In the region of the mesonephros, the mesomers detach themselves from the rest of the somite and fuse to form a continuous mesonephric blastema, in which swellings are seen. These are the mesonephric blastulae, — the fundaments of the mesonephric units. In a few cases they were found to correspond in number to the mesomers from which the blastema was derived (that is, are metameric in arrangement), but usually there is more than one to each somite. The distinction into swellings of the " first order " and those of the " second order," as observed in Amblystoma, cannot be made in Rana. The blastulae differ from those of Amblystoma in that they are (or seem to be) usually joined to each other by their tapering ends.

2. The dorsal sets of units develop from a fundament derived from the blastula of the primary unit, essentially as in Amblystoma. This fundament, however, instead of being early cut off from the primary blastula, retains its connection. When the development of the primary blastula is completed, this connection is found to be with its Bowman's capsule. As in Amblystoma, there seems to be one secondary unit for each primary, one tertiary for each secondary, etc. The number of dorsal units communicating with the collecting trunk of each primary decreases cephalad at such a rate that the most anterior primary may not possess even a secondary unit. There is not, however, as in Urodela, an extended region in which primary units are alone present.

3. The most striking peculiarity in the development of a mesonephric unit in Anura is found in the behavior of the outer tubule, — a peculiarity already observed by several authors. Each of the earlier outer tubules arising from the primary units grows out from the blastula in the manner described for Amblystoma. Shortly after its distal end reaches and coalesces with the peritoneum, its proximal end severs its connection with the rest of the unit and opens into a vein. Later in life many additional outer tubules appear. In *structure*, these are similar to the first ones, but their *origin* is quite different, and difficult to determine.

They seem to arise either (1) from a splitting of the first set, or (2) from independent evaginations of the peritoneum, or from both.

4. In regard to the *order of appearance* of the primary units, the usual statement that it progresses from behind forward, is incorrect (at least for Rana sylvatica). The majority of the primary units, those occupying all except the extreme ends of the kidney, *appear* simultaneously, but they *develop* from *behind forward.* Later, a few are added at both posterior and anterior ends of the series.

5. As in Amblystoma, the antero-posterior extent of the germ cells becomes much reduced as development proceeds.

III. Development of the Müllerian Duct.

The point of greatest phylogenetic interest in the development of the Müllerian duct, its relation to the Wolffian duct, is still in dispute. There seems no doubt that in elasmobranchs the greater part of it is derived from the Wolffian duct, and a similar origin has been claimed by the latest investigators for a portion of it in birds and mammals. In regard to the Amphibia, statements have been very contradictory, the Müllerian duct being described sometimes as developing independently of, sometimes in connection with, the Wolffian duct. Consequently my attention has been especially directed to this point.

Another problem, the solution of which may help in deciphering the phylogenetic history of the Müllerian duct, is the mode of formation of the *ostium abdominale.* In elasmobranchs, the ostium is said to be formed by a fusion of several of the pronephric nephrostomes. In birds and mammals, on the contrary, it would seem that it is formed by the coalescence of several evaginations of the coelomic epithelium, independently of the nephrostomes. In the Amphibia it has not been claimed, to my knowledge, that more than one coelomic evagination precedes the formation of the ostium. I, therefore, have given this question special attention in my investigations, in order, if possible, to throw some light on the suggested homology between the ostial evaginations of the higher vertebrates and the nephrostomes of the elasmobranch pronephros.

A. Amblystoma.

The Müllerian ducts of adult Amblystoma are rather simple structures, consisting of a pair of tubes, each having a single ostium abdominale opening into the body cavity, and a posterior outlet opening into the cloaca. The development of the Müllerian duct shows that the organ was originally more complex, for a portion of its fundament degenerates completely. In accordance with this fact, it is not surprising that the earlier stages are quite variable. This variability has made it seem best to describe individual larvae, — a rather cumbersome way, but one which obviates the necessity of constantly mentioning exceptions. To a certain extent, the larvae successively described represent successive stages; that is, they are so arranged that each one shows, *on the whole*, an advance in oviducal development over the preceding one, although in some particulars it may be less advanced.

Larva I, 23 mm.

In order to understand the early development of the Müllerian duct, it is necessary to follow the successive changes in the body cavity.

In a larva in which no sign of the duct is yet present, a cross-section cutting the anterior portion of the pronephros shows no body cavity, the entire region being filled with loose mesenchymatous tissue. A section a little farther back, passing through the first nephrostome (Fig. 31, Plate 3), shows three divisions of the body cavity on each side of the body: (1) A ventral division (*coel. v.*) at the side of the pericardium (*pi'cr.*); (2) a dorsal division, the glomerular cavity (*cav. glm.*), into which the nephrostomes open and the glomus protrudes; and (3) a cavity between these two, which I shall call the sub-glomerular cavity (*cav. sb'glm.*). At the stage represented by the larva under consideration, the sub-glomerular cavity is recognizable for only two or three sections. It is really but a shallow anterior outpocketing of the glomerular cavity, or, from another point of view, it may be said that there is a narrow shelf of tissue (*tab.*) which juts out from the anterior wall of the glomerular cavity, extending from the alimentary tract on the median side to a region just ventral to the pronephros on the lateral side. For convenience, I shall refer to this tissue simply as the "*shelf.*" Following the sections caudad, one finds that the glomerular and sub-glomerular cavities soon become confluent. The single cavity thus formed remains distinct from the ventral body cavity as far back as a point posterior to the pronephros. Figure 37 (Plate 3) taken from a section passing through the posterior part of the glomus on one side of the body (the right, in the figure left) and through the second nephrostome on the other, shows the dorsal (*coel. d.*) and ventral portions of the body cavity (*coel. v.*) separated by tissue joining the lung to the body wall (*pn. + par.*).

Larva II, 24 mm.

The condition of the body cavity in this larva is essentially as in Larva I, with the exception that the "shelf" has become more pronounced, now separating the glomerular cavity (Fig. 32, Plate **3**, *cav. glm.*) from the sub-glomerular cavity (*cav. sb'glm.*) as far back as halfway between the two nephrostomes, instead of for a distance of only two or three sections.

In Larva I the epithelium of the nephrostomes was thickened.[1] In Larva II the thickening of the lower lip of the anterior nephrostome is continued as a band ventrad over the face of the pronephros on to the shelf, where it turns caudad. This is shown in Figure 32 (*tae. e'th. α*), which is from a section passing immediately posterior to the first nephrostome, whose cilia (omitted by the lithographer) occur in the dorso-lateral angle of the glomerular cavity. When this band reaches the posterior limit of the shelf it passes below it for a very short distance on the lateral wall of the body cavity, turning slightly forward (Fig. 33, *tae. e'th. γ'*). It is as if the band had at first grown directly ventrad from the first nephrostome and had then been pushed backward by the caudad growth of the shelf (compare Fig. *I*, p. 88).

Larva III, 21.5 mm.

There is in this larva a slight but important advance over Larva II. The thickening of the ventral lip of the first nephrostome is distinctly accentuated to form a small thick disk (Fig. 36, *evg. 1*). From this the band runs ventrad, inclining somewhat caudad, then caudad until free of the shelf, then cephalad, precisely as in larva II. A slight thickening below the ventral lip of the second nephrostome is observable on one side of the body.

Larva IV, 44 mm.

This larva shows the beginning of a process which seems to be preparatory to the degeneration of the pronephros; that is, a compression of the pronephros and glomerular cavity in a dorso-ventral direction (Figs. 38, 39, Plate **4**). The glomus is also affected, becoming more and more attenuated. In Larva III it was confined to the space between the two nephrostomes, while in this larva it extends anterior to the first and posterior to the second. With the compression of the glomerular cavity, that portion of it which lies at the mouth of the first nephrostome becomes partially cut off to form what I shall call the "*nephrostomal cavity*" (Fig. 39, *cav. nph'stm.*). Whether this small cavity has any ontogenetic or phylogenetic significance, I do not know. It becomes more and more completely cut off in later stages, only to disappear entirely at a still later stage. The first nephrostome opens into the nephrostomal cavity four sections anterior to the point marked *cav. nph'stm.* in Figure 39. In the ventro-lateral angle of the nephrostomal cavity may be seen the thickened band already described for previous stages. It runs caudad and, just behind the point where the nephrostomal and glomerular cavities become confluent, passes out on to the shelf (Fig. 38, *tae. e'th. α*). Here it is joined by a similar band from the second nephrostome (*tae. e'th. β*). The section figured cuts this band just anterior to the point where it passes out to join the anterior band. The shelf extends much farther back than formerly (16 sections posterior to the 2nd nephrostome). The thickened band runs downward around its posterior edge and then forward along its ventral surface, — that is, along the dorsal wall of the sub-glomerular cavity. The extent and position of the band are represented in Figure *J*, p. 88.

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[1] The term "thickening" is somewhat of a misnomer. The most conspicuous feature is often not so much a thickening of the epithelium as a crowding together of the nuclei, which stain more darkly than those of the surrounding tissue.

In another larva, otherwise like the one last described, there is a slight accentuation of the thickening of the lower lip of the *second nephrostome*, similar to, but smaller than that of the next stage, which is shown in Figure 40 (Plate **4**). In neither of the larvae just described, however, has the thickened disk of the first nephrostome appeared, although it was present in Larva III.

Larva V, 41 mm.

In this larva the local thickenings near the first and second nephrostomes have both become quite pronounced (Fig. 41, *evg. 1*, and Fig. 40, *evg. 2*). The anterior one (Fig. 41) is not directly ventral to the first nephrostome, but lies behind it, for the nephrostome opens into the dorso-lateral angle of the nephrostomal cavity (*cav. nph'stm.*) eight sections anterior to the one figured. The nephrostomal cavity extends farther caudad than in previous larvae and its anterior end has become so narrow that it forms a postero-ventral continuation of the nephrostome, so that it is difficult to discern where the one ends and the other begins. At *tae. e'th. α* (Fig. 40) the band from the first nephrostome is shown.

Larva VI A, 40 mm.

The nephrostomal cavity is now still more completely cut off from the glomerular cavity than in Larva V. Figure 42 shows the section in which it becomes confluent with the glomerular cavity. The thickened disk which was seen in its ventro-lateral angle in the preceding larva (Fig. 41, *evg. 1*) is now seen to have evaginated to form a thick-walled pit (*evg. 1*, Fig. 42). In the evagination figured (from the right side of the body) there is a tendency toward a spiral coiling, a common but not universal feature in these anterior evaginations. The open lumen does not extend very far into the cell-mass, but the direction of its continuation is indicated by the arrangement of the nuclei. At the deep end of the spiral arises a duct (Fig. 43, *dt. 1*), which runs caudad for some sections, dwindles to a cord, and disappears (Fig. *K*, *dt. 1*). At the point of origin of "duct 1" a cord starts *cephalad* and continues for several sections. For the sake of convenience I shall call this the *precoelomic duct* (*dt. pr'coel.*, Fig. *K*). Its position at a later stage is shown in Figure 48, *dt. pr'coel.*

On the left side of the body, the first evagination is similar to that on the right side, with the exception that it arises more dorsally, from the middle of the lateral wall of the nephrostomal cavity (in a position corresponding to that of the thickening shown in Figure 41), and is a deep, *straight* pit with a wide lumen, much like the one shown in Figure 49.

From the mouth of the evagination the thickened band runs back (Fig. 43, *tae. e'th. a*) and out on the shelf, just as in previous stages, there to be joined by the band from the second or posterior nephrostome. The local thickening of the band from the second nephrostome is very conspicuous, but has not as yet evaginated. Figure *K* (p. 88) represents diagrammatically the condition of the evagination, etc., at this stage.

Larva VI B, 42 mm.

The anterior evagination on one side of this larva is similar to those in Larva VI A. On the other side it is very small, whether just arising or already degenerating, it is impossible to decide. An important advance over the preceding larva has been gained in that the thickened disk ventral to the *second nephrostome* has also evaginated both on the right and on the left sides of the body. These posterior evaginations, which, as will appear later, are the permanent ones, are not so deep as the anterior ones. Figure 44, *evg. 2*, shows that of the left side of the body and is drawn to the same scale of magnification as the anterior evagination (*evg. 1*, Fig. 42). It is a simple outpocketing, which is continued caudad, first as a duct, then as a cord which soon dwindles and disappears. Figure 53 shows this cord (*dt. 2*), more highly magnified, eight sections behind the evagination shown in Figure 44, and three sections anterior to its distal end. As the figure shows, it is entirely free from the Wolffian duct (*dt. Wf.*).

Along the course of this posterior duct the peritoneal epithelium is slightly thickened (Fig. 53, *crs. o'dt.*). Posterior to the distal[1] end of the cord, this thickening accompanies the Wolffian duct mediad and then caudad, and is continued as that enigmatical welt which lies near the Wolffian duct throughout its entire length and has been called the "Tubenleiste" by German authors (see Fig. 54, Plate 5, *crs. o'dt.*). Its appearance always precedes the formation of the Müllerian duct, and Wilson ('94), Semon ('92), and others have seen in it the fundament of that structure. I cannot find that it has anything to do with the development of the essential part of the Müllerian duct, — the epithelial lining. It often seems, however, at least in the anterior region of the body, to be proliferating cells to form the outer layers of the duct. The "Tubenleiste," or oviducal welt, as I have termed it, later disappears entirely.

Larva VII, 35 mm.

The pronephros of this larva shows undoubted signs of degeneration. The first and second nephrostomes have drawn quite near to each other, a very common condition during degeneration. Both pairs of nephrostomes have migrated slightly caudad: the first on one side, and the second on the other have become closed, and remain connected with the peritoneum merely by cords. With the closure of the first nephrostome, the associated nephrostomal cavity is obliterated.[2] This obliteration of the nephrostomal cavity does not affect the anterior evagination, however, as it has already moved out of the cavity by a migration caudad. (Compare Fig. *L*, p. 88.)

The conditions of the evaginations on the two sides of the body are as follows: On the right side the first nephrostome and nephrostomal cavity are still present. The anterior evagination is behind them; it is still massive and sends a cord cephalad (the pre-coelomic duct) a distance of some fourteen sections.[1] Just anterior to the evagination, this cord shows, by the perfectly radial arrangement of its nuclei, that it is really a potential duct. In passing forward this radial arrangement is soon lost, but the cord remains very conspicuous and runs directly cephalad, deeply buried in the pronephros. Figure 48 (Plate 4, *dt. pr'coel.*) shows a cross-section of the cord five sections posterior to its cephalic end. The arrangement of cells in the form of loose concentric layers is very characteristic of this pre-coelomic duct, as well as of the *posterior* duct of the anterior evagination and, to a less degree, of the duct from the second or posterior evagination.

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[1] I use the term "distal" to designate the portion *farthest from the origin.*

[2] It would seem that the obliteration of the nephrostomal cavity takes place not by a flattening out of its walls to merge with the general epithelium of the glomerular cavity, but rather by the closure of its mouth, followed by an actual dissolution of the walls, similar to that which takes place in the nephrostomal canals and pronephric tubules. If this is true, it forms a suggestive parallel to the process in Rana (see page 90).

The posterior duct of the anterior evagination extends back only a few sections. I am convinced that this is not due to its never having been formed, but to its early degeneration, for the duct from the second evagination is in many places accompanied by a second duct, and finally sends off a free cord, which runs parallel with it for some distance and then ends. It would seem, then, that the duct from the first evagination had reached back to, and fused more or less completely with, the duct from the second evagination, and that it had then almost wholly degenerated between the two evaginations. I lay special stress on the condition on this side of the body, because I think it explains a puzzling condition on the opposite side.

The first (anterior) evagination on the *left* side sends back a cord which reaches the level of the second evagination and is there lost sight of (compare Fig. *L*, p. 88). The second evagination sends back its cord for about a somite and a half. Then for a distance there is *no sign* of one, until suddenly it *reappears* again for a short distance. This condition made me think, at first, that Wilson and others were right in believing a part of the duct to be formed *in situ* from the cells of the oviducal welt. I am convinced, however, from the condition on the right side described above, that this isolated piece is simply a fragment of the cord from the *first* evagination which has escaped degeneration. This belief is supported by the fact that similar isolated fragments of the first duct may remain, even in much later stages, between the two evaginations, where there can be no doubt as to their origin (see *dt. 1*, Fig. 52, Plate **4**; and, for description, p. 87).

To sum up the condition of the fundament of the Müllerian duct at this stage: The thickened disks ventral to the first and second nephrostomes have evaginated to form open pits, which have begun to migrate caudad, especially the posterior one. From the anterior one a thickened epithelial band runs caudad, then ventrad around the free end of the shelf (which now extends some distance back of the pronephros), and then forward on its ventral surface. Both on the ventral and on the dorsal side of the shelf the band shows a tendency to slip off, as it were, on to the lateral body-wall, so that in its backward course it now passes close to the second evagination, thus reducing the length of the band which connects it with the second evagination.

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[1] On the left side of the body it extends cephalad for 19 sections.

From both first and second evaginations ducts (or cords) run caudad. That from the first fuses more or less completely with that from the second, and shows a tendency to degenerate between the two evaginations. There is an additional cord extending *cephalad* from the first evagination, which has no known ontogenetic meaning, but *may* have an important phylogenetic significance. As this cord (which is sometimes met with in later stages) completely degenerates without participating in the formation of the Müllerian duct, I shall leave it out of the description of subsequent stages. Finally, the sub-glomerular cavity has begun to extend itself cephalad beyond the glomerular cavity.

Larva VIII, 55 mm.

In this and later stages the distortion suffered by the degenerating pronephros gives rise to much variation in the relative position of its parts. Sometimes, as in the present larva, the glomus, narrowly confined in its cavity, lies wholly anterior to the tubule-mass of the pronephros. It may, however, lie opposite, or almost wholly behind the pronephros. Some or all of the nephrostomes may remain open until a much later period. Generally, however, the anterior ones at least are closed, and may even be wholly severed from the peritoneal epithelium. A like variation is found in their position. In general there is a tendency toward a caudal migration. In one case the first nephrostomal tubule on one side of the body had in its migration passed the second nephrostome and ended posterior to it near the peritoneum. The nephrostomal cavities are usually absent.

The condition of the evaginations in this larva differs from that in Larva VII, only in that they have all migrated caudad (Fig. *M*, p. 88). An important result of this migration is that it has brought the posterior evagination into a position posterior to the free edge of the shelf.

With the dorso-ventral compression of the pronephros (probably due to the growth of the stomach), the anterior evagination no longer lies at a lower level than the nephrostomes. Instead it lies at the same level, or even more dorsally than they. As in the preceding larva, the duct from the anterior evagination disappears in that part of its course which is anterior to the second evagination, but the duct from the latter is in places double, showing that it has been formed by a fusion of the two ducts. The posterior duct on both sides of the body becomes curiously attenuated a short distance behind its origin, so that it is reduced in cross-section to two or three cells, but is always clearly marked off from the surrounding tissue by its sheath.[1] Farther back it again becomes a well-defined tube, which extends for some distance alongside the Wolffian duct. Figure *M*, page 88, illustrates this and the next stage.

Larva IX, 50 mm. — Gills reduced to stumps.

This larva is interesting from the fact that, although older than any of the preceding, the duct from the anterior evagination (both right and left) has not suffered any degeneration. It (*dt. 1,* Fig. 51, Plate **4**) passes the second evagination and, after running parallel to the duct from the second evagination for some distance, approaches and fuses with it. The beginning of this fusion is shown in Figure 50 (*dt. 1*, *dt. 2*). The single duct thus formed continues caudad for some distance along the Wolffian duct. On one side of the body it ends independent of the Wolffian duct and enclosed in its own sheath. On the other side it approaches the Wolffian duct until the two are enclosed in a common sheath and then, applying itself to the Wolffian duct, disappears apparently by fusing with it. Figure 51 shows the thickened band (*tae. e'th.* α), which passes back from the first evagination, around the posterior edge of the shelf (*marg. p. tab.*) and forward again in the sub-glomerular cavity (*tae. e'th.* γ').[2] Two sections behind the one figured, the shelf separates along the line *marg. p. tab.* from the lateral body-wall. Where this occurs, the two portions of the thickenings *tae. e'th.* α and *tae. e'th.* γ' are seen to be continuous. In the same section the ridge containing the Wolffian duct and fundaments of the Müllerian duct becomes well separated from the lateral body-wall (as in Figure 50) by an infolding, whose position is indicated in Figure 51 by the groove *sul.*

[1] In poorly preserved specimens this attenuated part of the duct might easily be overlooked. This condition may account for the conception of some authors that the duct is not developed continuously with the ostium.

[2] By comparing Figure *J* with *M* it will be understood that the only part of the epithelial band now remaining on the upper side of the shelf is that designated by α, γ having merged with γ' by being brought beneath the shelf.

Larva X, 53 mm. — Gills reduced to stumps.

On the right side of this larva the anterior evagination has almost totally disappeared, as well as that part of its duct which was anterior to the second evagination (Fig. *N*, p. 88). The duct from the second evagination divides, however, almost immediately into an open tube and an irregular cord. These soon reunite to form one large duct with two lumina, which finally merge into a single lumen. The condition of the distal (caudal) end of this duct I shall describe presently. On the left side of the body both evaginations are still present with their ducts, which at first are widely separated, then approach and have a common sheath. They do not unite completely until they reach the region of the *fiftieth* section back of the second evagination. The resultant duct fuses with the Wolffian duct. The second evagination is so far back that it lies in the ridge whose formation was traced in Larva IX. This causes the thickened band and the duct from the second evagination to form an almost straight line, as shown in Figure *N*.

The distal end of the Müllerian duct on the right side of the body presents as strong evidence as any I possess, that a portion of the Müllerian duct may fuse intimately with, and possibly take cells from, the Wolffian duct. As the question of the participation of the Wolffian duct in the formation of the Müllerian duct has been so much discussed and is of such great theoretical interest, it seemed best to make drawings of a number of *consecutive sections* and thus give the reader a better opportunity to judge for himself as to the evidence.

As was previously stated, the single duct, formed by the fusion of those from the two evaginations, runs for a certain distance close to the Wolffian duct, but enclosed in its own sheath; that is, a layer of connective tissue separates it from the Wolffian duct. Back of this the duct approaches the Wolffian duct and this connective-tissue layer is interrupted, thus allowing the Müllerian duct to apply itself to the Wolffian. Figures 54 to 63 (Plate **5**) show ten consecutive sections immediately posterior to the point where this juxtaposition takes place.

In Figure 54 is seen a mass of tissue extending from the Müllerian duct (which a few sections farther forward is quite free and in cross-section regularly rounded) to apply itself to the Wolffian duct. The two following sections (Figs. 55 and 56) show the Wolffian duct distorted in such a way that its median wall is continuous with this mass of cells. In Figure 57 the mass is quite distinct from both ducts, and the Wolffian duct has regained, to a large extent, its typical shape. In Figures 58 and 59 the mass is seen to have again attached itself to the Wolffian duct and partially (Fig. 59) to the Müllerian duct as well. The two following sections (Figs. 60, 61) are especially significant, in that they show a cell which has just divided into two daughter-cells, one of which (Fig. 60) seems to constitute a part of the wall of the Wolffian duct, the other (Fig. 61) a part of the cell-mass which in turn is quite intimately connected with the Müllerian duct. In the two following sections (Figs. 62 and 63) the cell-mass nearly disappears, a portion of it allying itself with the Wolffian duct, the remainder forming a separate strand closely applied to the Müllerian duct. This and the Müllerian duct now become separated from the Wolffian duct by a layer of connective tissue. The strand just described is more or less distinctly differentiated from the mass of the Müllerian duct for some ten additional sections. It then separates entirely from that duct and, passing again through a gap in the connective tissue layer which separates the two ducts, fuses with the Wolffian duct. The posterior end of this fusion is shown in Figure 64, where the lumen of the Wolffian duct is seen to present a slight outpocketing ventrally, which represents the posterior end of the lumen of the strand just described. Posterior to this point, the Müllerian duct extends free for some distance and then ends.

The pictures presented by these sections may be interpreted in two ways: Either the duct had fused with the Wolffian duct and is now drawing away from it, leaving strands of tissue still connected with both ducts, or the Wolffian duct is participating in the formation of the Müllerian duct by proliferating cells which form irregular masses and strands of tissue extending from the former to the latter. The second view is supported by the presence of the dividing cell shown in Figures 60 and 61.

My conviction that the Wolffian duct participates, to a small extent, in the formation of the Müllerian duct, rests not only on the fact that at certain stages there is always at least a juxtaposition of the two, but also on the additional fact that, where this occurs, cell-division is frequently taking place in the wall of the Wolffian duct. This formation of new cells at a time and place where the Wolffian duct is functionless and about to degenerate is surely very significant.

Larva XI, 49 mm. — No gills.

The most anterior sign of the fundament of the Müllerian duct in this larva is the thickened band, which begins anterior to the pronephros and extends caudad on the dorso-lateral wall of the sub-glomerular cavity

(Fig. 49, Pl. 4, *tae. e'th.* γ') along the line where the body-wall and shelf join each other. As the second evagination — which I shall henceforth call the *ostium* — has migrated caudad until it is well out of the glomerular cavity, the thickened band no longer needs to make a bend cephalad to reach it. Instead it runs straight back, becomes a groove, then by further folding a tube, and finally continues caudad as the Müllerian duct, which is now simple and shows no signs of its double origin (see Figure *N*, where, however, it is represented as double). Figure 52, *tae. e'th.* γ', represents the band after it has passed the posterior limit of the shelf and a short distance anterior to the ostium.[1] On the left side of the body the anterior evagination and a portion of its duct are retained in a remarkable degree of perfection. Figure 49, *evg. 1*, shows in section this first evagination, which has lost all connection with the thickened band, as shown in Figure *N*. Its duct is represented by a continuous cord, the nuclei of which are arranged radially, which disappears, however, before it reaches the ostium (or second evagination). On the right side both the first evagination and its duct have disappeared, with the exception of a portion of the latter, which, though only a few sections in length, is well formed (Fig. 52, *dt. 1*). The retention of this detached bit of the anterior duct is important, as it lends strength to the assumption made in the description of Larva VII (page 82), that the detached portion there seen was a survival from the degenerating duct of the first evagination.

The grooved condition of the thickened band anterior to the ostium is especially noteworthy. It signifies that the process whereby the ostium is transferred to a more and more anterior position has already begun. This process consists in a longitudinal folding of the thickened band, followed by a fusion of the edges of the groove thus formed, which, beginning at the ostium, advances cephalad.

Larva XII, 55 mm. — Neither gills nor gill-slits.

This is the latest stage which I shall describe. It is essentially like the preceding, with the exception that all traces of the anterior evagination and its duct have disappeared. The glomerular cavity is much reduced in diameter. It has the form of a long narrow *tube* (Fig. 45, Plate 4, *cav. glm.*) nearly filled at its anterior end with the now thread-like glomus. It still connects with the sub-glomerular cavity (now synonymous with the body cavity, the pneumo-parietal fusion having

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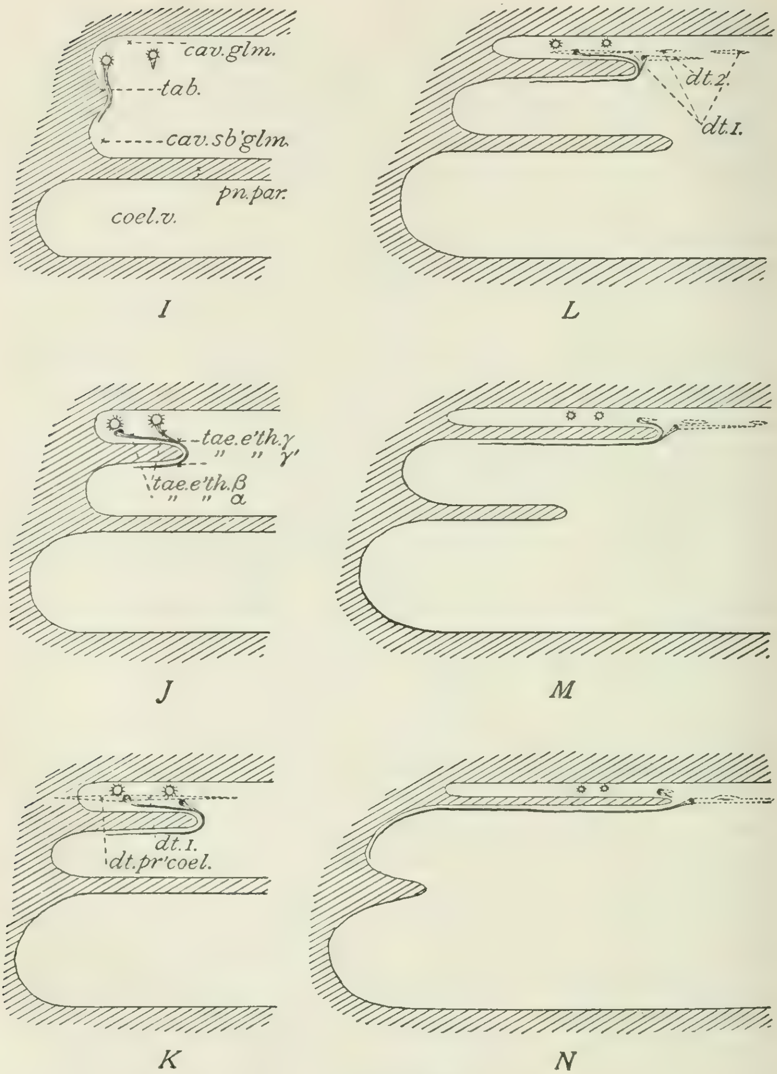
[1] At earlier stages, this portion of the band was designated as β.


cav.glm.
tab.
cav.sb'glm.
pn.par.
coel.v.
I
dt.2.
dt.1.
L
tae.e'th.γ
" " γ'
tae.e'th.β
" " α
J
M
dt.1.
dt.pr'coel.
K
N

Figures *I–N.*

Six diagrammatic figures to show the relation, at various stages, of the Müllerian evaginations, epithelial bands, and nephrostomes to the divisions of the body cavity in Amblystoma. A parasagittal cut, to the right of the stomach, exposes the body-wall. The head is to the left.

In Figure *I* the epithelial band from the first nephrostome is being deflected caudad by the growth of the shelf (*tab.*), which is destined to divide the upper portion of the body cavity into a glomerular and sub-glomerular cavity. (*See opposite page.*)

disappeared) by a narrow slit (Fig. 46, near *marg. p. tab.*), that is, the posterior margin of the shelf (Fig. 45, *tab.*) is still free. Degenerate pronephric tubules (Fig. 45, *tbl. pr'nph.*) are recognizable. The grooving or folding of the thickened band (which is supported by a fold of the peritoneum) now extends for a long distance cephalad from the ostium (Fig. 47, *evg. 2*), instead of for only a few sections, as in Larva XI. It reaches an entire somite anterior to the position of the adult ostium, which is opposite the posterior end of the fourth vertebra. I believe this seemingly superfluous anterior portion is utilized in forming the *downward* curve of the anterior end of the adult duct, which, instead of opening near the dorsal line of the body cavity, curves downward and outward around the base of the lung to its final position in the ventral portion of the body cavity. This downward curving seems to be brought about by the progressive restriction of the body cavity anteriorly.

Figure 47 shows the ostium (*evg. 2*) two sections anterior to the point where it becomes closed off from the body cavity. For thirteen sections behind that point, the duct shows two lumina. This may be regarded as evidence of its double origin.

There is a rather constant difference in the formation of the Müllerian duct on the two sides of the body. On the left side, the thickened band lies more laterally, and the grooved condition always extends farther forward than on the right side. I have been unable to detect any difference in the formation of the duct in the two sexes. If, however, a larger number of specimens were examined with this special point in view, minor differences might perhaps be found.

The formation of the "shelf," which is also described by Hoffmann ('86) and Gemmill ('97) as existing in Triton, seems to me interesting. It has generally been held that the glomerular cavity in the Urodela is of little significance, it being a sort of accidental consequence of the temporary fusion of the lung with the body-wall. We see that there exists in Amblystoma a glomerular cavity of an independent and more

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In Figure *J* the first evagination (the black dot beneath the first nephrostome) has appeared. The epithelial band may be divided into four regions: (1) a portion (*tae. e'th. α*) running back from the first evagination; (2) one (*tae. e'th. β*) from the second nephrostome, joining (1); (3) their combined prolongation (*tae. e'th. γ*) above the shelf; and (4) the same (*tae. e'th. γ'*) extending forward beneath the shelf.

In Figure *K* the second evagination has appeared and the first has sent the pre-coelomic duct (*dt. pr'coel.*) forward and a duct (*dt. 1*) backward.

Figures *L*, *M*, *N* scarcely need further explanation. The second evagination sends back a duct (*dt. 2*) which fuses with portions of the duct from the first, the remainder of this latter duct, as well as the epithelial band from the first evagination, degenerating. The extent of the fusion of the lung with the body-wall (Fig. *I*, *sep. pn. par.*) is gradually diminished in the successive stages.

permanent character. It is true that it becomes well established only during the degeneration of the pronephros, but this fact is of little significance, from a phylogenetic standpoint, to those who would homologize the pronephric glomerular cavity with those of the mesonephros.

B. Rana.

As is well known, the three nephrostomes in Anura open separately into a portion of the body cavity which is partially cut off from the general body cavity by a fusion of the lung with the lateral body-wall, that is, with the surface of the pronephros. This pocket contains the glomus and is called the glomerular cavity. In Rana sylvatica, immediately before the degeneration of the pronephros, the glomerular cavity is forced to a more nearly median position and much reduced in size by the growth of a shelf[1] (Plate **5**, Fig. 65, *tab.*) comparable with that found in Amblystoma. As the pronephros retains its original position, the nephrostomal tubules are compelled to elongate in order to retain their connection with the glomerular cavity. The three nephrostomal tubules, which originally opened quite far apart, converge toward a common point, so that they finally acquire a common opening, which I shall term the nephrostomal vestibule, or *common nephrostome.* Figure 65 shows this common nephrostome (*vst. nph.*), and also the second nephrostomal tubule (*tbl. pr'nph.*) one section anterior to its opening into the common nephrostome. This condition, which, to my knowledge, has never been mentioned in descriptions of the degeneration of the pronephros, may, of course, exist only in R. sylvatica. In that form it exists, almost without exception, in all the larvae over twenty-five millimetres in length which I have examined. The common nephrostome bears some resemblance to, and may be homologous with, the nephrostomal cavity in Amblystoma. Just as the first nephrostome in Amblystoma was finally cut off by the closure of the nephrostomal cavity, so here the three nephrostomes become cut off from the coelom by the closure of the common nephrostome.

To anticipate a little, the later history of this common nephrostome is illustrated by Figures 68–77 (Plates **6**, **7**). It gradually becomes deeper and narrower until it resembles the ordinary nephrostomes. Like them it is ciliated, and may have been mistaken for the third nephrostome, which is generally described as persisting to a late stage. In Figure 68 is seen the end of the first nephrostomal tubule, in Figure 69 its opening into the common nephrostome. The connection of the latter with the coelom is shown in Figures 70, 71, and 72. The second nephrostomal tubule is shown in the same three figures, and the third in Figures 75–77. The first nephrostomal tubule comes into the common nephrostome from an anterior direction; the second from a point directly laterad, and the third curves dorsad and then cephalad.

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[1] The shelf in Rana sylvatica extends caudad but a short distance and is a temporary structure. Very often the lung, which early loses its connection with the lateral body-wall, is fused with the ventral side of the shelf.

The first sign of the fundament of the Müllerian duct appears in the form of a thickening of the peritoneal epithelium of the pronephros at a little later stage than the one from which Figure 65 was taken. It will be remembered that the peritoneal evaginations in Amblystoma which formed such an important part of the fundament of the Müllerian duct, arose just ventral to the two nephrostomes. The conclusion is obvious that the nephrostomes may in some way determine the position and number of these evaginations. If that is true, we should expect to find three evaginations in Rana, one beneath each nephrostome. But the fundament of the Müllerian duct does not arise until after the migration of the three nephrostomes to a common region, so that the tissue immediately ventral to them is now probably represented by tissue just ventral to the common nephrostome. Bearing this in mind, as well as the fact that the embryological processes in the highly specialized Anura are often hurried and obscure, as compared with those in the Urodela, I was not surprised to find that the theoretical three evaginations are generally represented by a single irregular mass of cells proliferated from the peritoneal epithelium. This mass is always *elongated antero-posteriorly*, and lies ventral to the common nephrostome. It usually shows more than one obscure evagination from the coelom extending into it. Only once did I find it represented by one large, well-formed evagination. I searched carefully for evidence that the three evaginations are not purely theoretical, and was fortunate enough to find one case in which the complete fusion of the three nephrostomes had been retarded until after the appearance of the fundament of the Müllerian duct. In this larva, on one side, the two anterior nephrostomes are fused, but the third still opens separately at some distance behind the common opening of the anterior ones. Ventral to the opening common to the first and second nephrostomes, there is a distinct evagination of the peritoneum (Plate 5, Fig. 66). From this a thickened band runs ventrad and cephalad over the face of the pronephros along its line of junction with the shelf. *Ventral to the third nephrostome is a thickened disk*

(Fig. 67), similar to those which formed the evaginations in Amblystoma. From this disk a thickened band runs ventrad and cephalad to join that from the first evagination. The opposite side of the body presents the normal condition of a single irregular evagination ventral to the common opening of the three nephrostomes.

It was stated above that even where the three nephrostomes have fused, the fundament of the Müllerian duct may show signs of being formed of separate proliferations or evaginations. I have represented ten successive sections through such a fundament in Figures 68–77 (Plates **6**, **7**).

In Figure 68 the proliferation (*evg. 1*) may represent the first evagination, although it is also possible that it is the posterior end of the fold of the thickened band which is described below. If it is really the first evagination, the mass of cells marked *dt. 1*, in Figures 69 and 70, would seem to represent the duct which in Amblystoma extends caudad from the first evagination. In Figure 70 the epithelial thickening shows no evagination or special proliferation. In Figures 71 and 72 the cell proliferation immediately beneath the common nephrostome is well marked and takes the form of a distinct evagination, whose lumen is shown in Figures 73 and 74 (*evg. 2*). These sections also show a group of cells (*dt. 2*) which may represent the duct from this second evagination. Owing to the thickness of the sections, this group of cells is longer than one would at first imagine. Figure 75 passes through the posterior margin of the second evagination. Directly ventral to it is the thickest point in the peritoneal epithelium, which marks the level at which the third evagination takes place. The opening of the third evagination is shown in one section only (Fig. 76, *evg. 3*). The next section (Fig. 77, Plate **7**) passes through its posterior prolongation, and in the two following sections (not figured) is seen its duct, which is free from the peritoneum. This duct dwindles to a cord, which is short and ends in contact with, but independent of, the peritoneal epithelium. This cord forms the Müllerian duct, but whether alone or with the addition of anterior ducts (by fusion, as in Amblystoma), I was unable to determine. I must also leave undecided the important question whether the developing Müllerian duct comes into relationship with the Wolffian duct or not. Two factors render the determination of these points very difficult: the fold which contains the ducts turns mediad behind the pronephros, so that it is cut very obliquely in cross-sections of the body; this fold, like the degenerating pronephros, is filled with densely packed lymph cells. That the Müllerian duct takes cells from the Wolffian duct in this region seems very improbable for the reason that by the time the former reaches this region the degeneration of the Wolffian duct has rendered it difficult of recognition if, indeed, it can be seen at all. Posterior to the point where the fold containing the ducts reaches its most nearly median position and turns again caudad, so that it is cut transversely, the Müllerian and Wolffian ducts are seen, in later stages, imbedded in the lymphoid tissue *at some little distance from each other.* The growth caudad of the Müllerian duct must be rather slow, for in a young female frog which had completed its metamorphosis and left the water, and in which the eggs were quite large and surrounded each by a distinct follicular layer, the Müllerian duct had not reached the mesonephros. On the other hand, the oviducal welt, in which it lies when developed, reaches as a well-defined ridge nearly to the cloaca, and is generally *widely separated from the Wolffian duct.*

To return to the history of the anterior end of the duct; as in Amblystoma, the adult ostium is not formed directly from any of the evaginations. Instead, the larval ostium is carried cephalad and ventrad, utilizing the thickened band which, from its first appearance, takes that course. The anterior extension of the Müllerian duct takes place at a much earlier stage in Rana than in Amblystoma, but is accomplished by fundamentally the same method, as follows: the thickened band which extends from the evaginations cephalad and ventrad becomes broader, and the dorsal edge folds downward as shown in Figure 79 (Plate 7).[1] The edge of the fold, which projects into the body cavity, becomes continuous caudad with the dorsal lip of an evagination which is probably the third, though it may be a composite of two or three evaginations. Beginning at this point, the free edge of the fold then fuses with the portion of the band which forms a part of the peritoneum, as shown in Figure 80, which is two sections posterior to the one represented in Figure 79. This bending down and fusion of the dorsal edge of the band with its ventral margin which begins in the region of Figures 79 and 80, continues cephalad and ventrad until the opening reaches the position of the adult ostium at the base of the lung. This process of the forward and ventrad shifting of the ostium begins about the time of the appearance of the fore legs.

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[1] Anteriorly, where the band takes a ventral direction, this dorsal edge naturally becomes anterior in position and hence folds over in a caudad direction. The section figured cuts the band where it has turned ventrad, and hence quite obliquely. The free edge of the fold, therefore, appears much broader than it would if it were cut perpendicularly.

C. Hyla.

After writing the above description of the development of the Müllerian duct in Rana sylvatica, I chanced upon a form, Hyla versicolor, which seemed to show conditions worthy of careful study. As the results tend to confirm the conception of the multiple origin of the Müllerian evagination in Rana sylvatica, which was expressed in the above description, I have allowed that to stand as it was written.

The peculiarity in the development of Hyla which makes the development of the ostium more easily intelligible than that of Rana lies in the fact that only the two posterior pronephric nephrostomes, instead of all three, fuse to form a "common nephrostome." At what age the fusion of the two nephrostomes takes place could not be determined, as it had already taken place in my youngest specimen, in which the hind legs were still quite inconspicuous. In this specimen the second and third primary nephrostomes empty at some little distance from the peritoneal surface into a ciliated common nephrostome.

The development of the Müllerian duct takes place at about the same stage, relative to the external signs of metamorphosis, as in Rana sylvatica. In the youngest stage which I shall describe it is evident that the pronephros has but recently ceased functioning, as degeneration of the tubules and Wolffian duct has not proceeded far. Already, however, those thickenings of the peritoneal epithelium which participate in the formation of the Müllerian duct are conspicuous. Their condition is as follows: near the anterior end of the body cavity a thickening of the epithelium is visible ventrally. This curves dorsad and caudad, growing thicker as it proceeds. When it reaches the first nephrostome it is continuous with a much more marked thickening surrounding that opening. Contrary to the condition in Amblystoma and Rana, it is the *dorsal* lip which is especially thickened, and, as will be seen later, it is this thickening (*evg. 1*, Fig. 81, Plate **7**) immediately dorsal to the nephrostome, which forms the anterior Müllerian evagination.

The first nephrostome opens anterior to the root of the glomus. The second — the *common nephrostome* — opens posterior to it. Along a line joining the first with the common nephrostome the general thickening of the surface of the pronephros is accentuated to such an extent that there is formed a conspicuous band of large cells. This band is almost invariably folded so as to form a groove or trough (Fig. 83, *tae. e'th.*), whose concavity is directed toward the coelom. A thickened disk dorsal to the common nephrostome is also visible (Fig. 82, *evg. 2, 3*). Posterior to the common nephrostome all traces of the peritoneal thickening disappear.

The further history of the various portions of this peritoneal thickening is as follows: the thickened disk dorsal to the first nephrostome evaginates to form a pit, which generally is slightly anterior to the nephrostome. Figures 87 to 93 (Plate **8**) represent seven sections through such an anterior evagination. Figures 87–92 represent successive sections; Figure 93 is three sections posterior to 92.

This evagination is evidently comparable with the anterior evagination of Amblystoma, and there are sometimes distinct signs of a proliferation of cells from its distal end extending in a posterior direction, which may be considered homologous with the "posterior cord of the anterior evagination" of Amblystoma. In fact, it seems probable that these cell-proliferations sometimes take on the form of more definite cords, for there is occasionally seen in later stages a distinct cord — or even duct — which begins back of the degenerating evagination and extends caudad, close beneath the peritoneum. It is possible that such cords are really portions of the degenerating pronephric tubules, but I think not, as my attention was called to them by the fact that, instead of being indefinite tubules with large, pale cells, like those seen in the rest of the degenerating pronephros, they are clear-cut, with cells staining very darkly and possessing little cytoplasm.

Whether or not these cords form a part of the Müllerian fundament, it is clear that, as in Amblystoma, the anterior evagination and its cord play no *essential* rôle in the formation of the Müllerian duct and are but relics of a past history — for both evagination and cord disappear entirely.

While the anterior evagination is disappearing, the thickening dorsal to the common nephrostome grows more pronounced and in turn evaginates. From its distal end cells are proliferated to form a cord, then a duct, running straight caudad. This is of course the Müllerian duct. Figure 84 (Plate **7**) shows such a posterior evagination (*evg. 2, 3*), the section being three sections posterior to the point where the second and third nephrostomal tubules (shown in the figure) are joined to the peritoneum by a cord of cells representing the degenerating common nephrostome. Figure 78 (Plate **7**) shows the posterior evagination at a later stage. Sections posterior to the one figured show that it is continued caudad as a duct which soon dwindles to a cord and then ceases.

In Hyla versicolor the question as to whether the more anterior portion of the Müllerian duct during its growth caudad takes cells from the Wolffian duct, is easily answered in the negative, for the Wolffian duct has generally degenerated nearly back to the region of the mesonephros before the Müllerian duct arises. In one case only was a small bit of the anterior portion of the Wolffian duct retained, and in that case the posterior end of the Müllerian duct, although near by, was clearly independent of it. What the relation of the two ducts is when the Müllerian reaches the undegenerated part of the Wolffian which extends along the mesonephros, I have not observed.

The processes which precede the establishment of the adult ostium show a close similarity to those described for Amblystoma. During the changes described above, the posterior evagination and its associated (degenerating) nephrostome have migrated caudad. The anterior evagination where traces of it remain, is seen to have retained its position near the root of the glomus. The posterior one, however, instead of lying a short distance behind that point, as formerly, has migrated sometimes as much as twenty sections, or about seventy-four micra, caudad. The thickened band, which was mentioned as connecting the two nephrostomes, being still present, has become greatly elongated. It is also much more conspicuous than during the earlier stages, partly because its cells have become higher, partly because the cells of the adjacent peritoneal epithelium have taken on a squamous form. The trough-like form of the band becomes more pronounced near the second evagination. Since the band is continuous with that structure, a progressive fusion of its edges, beginning at the second evagination, results in a displacement cephalad of the opening of the Müllerian duct, as described for Amblystoma. The approximation of the edges of the trough preparatory to their fusion is shown in Figure 101 (Plate 8). In the next section posterior to the one figured the trough is closed to form the Müllerian duct, which extends thence caudad. In this case the anterior migration of the opening has been but slight. The degenerating common nephrostome is only three or four sections posterior to the one figured and is *dorsal* to the opening of the duct. This is the only case I have seen in which the evagination is ventral to the nephrostome, and is important as an indication that the position of the evagination, dorsal or ventral to the nephrostome, is not important. On the other side of the body the evagination is dorsal, as usual.

This is as far as my material allowed me to follow the ostial development, but as the trough is continuous anteriorly with the thickening which curves ventrad, and as the adult ostium lies ventrally near the base of the lung, there can be no doubt that the rest of the development is essentially as in Amblystoma.

It will be remembered that I have claimed, on grounds of analogy, that the Müllerian evagination in Rana sylvatica represents three evaginations, and that evidence of at least two was not lacking. That evidence was twofold: (1) The cell-mass which proliferates to form the Müllerian duct sometimes evaginates at two (and possibly three) points. (2) In a case where the third nephrostome had not fused with the other two, there was present an evagination near the fused, anterior two, and a disk (representing an evagination) near the posterior, free one. It has been shown that in Hyla versicolor the anterior nephrostome is independent of the fused posterior ones, and that there is an evagination associated with the former as well as one with the latter. With two evaginations patent, I hoped to find evidences of a third by a careful study of the posterior one, which, theoretically, should consist of a combination of two. I have looked in vain for such evidence as was found in Rana. The posterior evagination, although often much elongated antero-posteriorly, never showed distinct signs of a separation into two pits. Fortunately, however, I found one case in which all three nephrostomes had remained distinct; that is, the two posterior had not undergone the normal fusion. As a parallel variation in Rana gave rise to a separation of the Müllerian evagination into two, it seemed probable that in this case three fundaments might be distinguished. In effect, the condition was as follows. Dorsal to the first nephrostome was a very conspicuous evagination. The second and third nephrostomes were far back of the first and quite near each other, the second being ventral and somewhat anterior to the third. Figure 85 (Plate 7) represents a section passing through the posterior edge of the second nephrostome and just anterior to the third (some cilia of the latter are cut). There will be noticed a rather marked thickening (*evg. 2*) dorsal to the second nephrostome. While not very conspicuous, it is so restricted, — so clearly marked off from the surrounding epithelium, — and resembles so closely in staining properties, etc., the disk which always precedes the formation of a Müllerian evagination, that there is no doubt in my own mind that it really represents a second evagination. The evagination associated with the third nephrostome is typical. It is shown in Figure 86 (*evg. 3*), which is from the second section behind the nephrostome.

It will be seen that in the development of the Müllerian duct, Hyla versicolor occupies a place midway between Amblystoma and Rana sylvatica. In the presence of well-defined evaginations in connection with *more than one* nephrostome, the oviducal development approaches that of Amblystoma. Furthermore, the caudad migration of the posterior evagination and the method of carrying the opening of the Müllerian duct forward again along a trough of columnar cells by a progressive fusion of the edges of the trough, is exactly similar to what was seen in Amblystoma. On the other hand, the fusion of nephrostomes allies the development with that of Rana. As in Rana the fusion of all three nephrostomes caused either a suppression or a combination of the Müllerian evaginations into one, so in Hyla the fusion of two nephrostomes reduces the number of evaginations to two.

D. Comparison with the Results of other Authors.

Perhaps the most satisfactory way to give an idea of the various views which have been held in regard to the development of the Müllerian duct in Amphibia is to give a brief résumé of those accounts which are most important, either from their completeness or the recentness of their appearance.

Urodela.

Fürbringer ('78) states that during the early stages in the degeneration of the pronephros of Salamandra maculosa — at the time when the gills are disappearing — its glomerular cavity becomes more extensively separated from the body cavity. Along the lateral margin of the glomerular cavity the peritoneal epithelium becomes thickened. This thickening spreads out laterally and "distally" (that is, caudad) from the pronephros and forms a part of the fundament of the Müllerian duct. At the same time, from the ventral side of that portion of the Wolffian duct which is immediately posterior to the pronephros, a solid cord of cells is cut off, which then gains a lumen and is transformed into a duct. The hollowing-out process begins proximally (cephalad) and progresses caudad. Its cephalic end then fuses with the thickening above described, and through it opens into the body cavity to form the ostium abdominale. Caudad the duct continues to be cut off from the Wolffian duct and to become hollowed out. Its caudal end dwindles to a cord and is lost in the ventral or lateral wall of the Wolffian duct. It may, however, *end free*, with a blunt point, this condition being observed but once.

If the development in Salamandra is like that in Amblystoma, it would seem that in the earlier stages Fürbringer mistook for the Müllerian duct the degenerating duct which passes backward from the first evagination. If he observed that the duct from the first evagination ended blindly in front, as it sometimes does in Amblystoma, and if he later found the Müllerian duct connected anteriorly with the second evagination, the impression would be natural that the ostium was formed secondarily. That he found no evagination at that early stage may be due to the fact that the first evagination degenerated before the second was formed — although I have never observed such a condition in Amblystoma. Or, it would be very easy to mistake the first evagination for a nephrostome. It must not be thought, however, that I criticise the observations of Fürbringer, or other authors, as necessarily incorrect. Since a certain controversy in regard to the development of the vasa efferentia in Rana, which had its origin in the fact that the observers were working on different species, we have been taught that it is not safe to take for granted that the details of embryonic development are alike even in the *same genus.*

In general Hoffmann ('86) confirms for Triton cristatus the observations of Fürbringer on Salamandra. After reaching the height of its development, the pronephros migrates to a more median and dorsal position and the two nephrostomes draw nearer together. The glomus, which originally occupied a position between the two nephrostomes, is brought to lie opposite the first. About the time when the gills begin to degenerate, the segmental duct splits longitudinally to form the Wolffian duct (dorso-median) and the Müllerian duct (ventro-lateral). The splitting begins anteriorly and progresses gradually caudad. Shortly after the first appearance of the Müllerian duct its anterior end forms the ostium abdominale by fusing with the thickened peritoneal epithelium, — generally lateral to, and on a level with, or just posterior to, the second nephrostome, sometimes between the two. This thickening, which is formed by the cells of the peritoneum taking on the columnar shape, existed "lateralwärts neben und zwischen den beiden Trichtern" before the appearance of the Müllerian duct. The backward growth of the duct is different in the two sexes. In the female its whole length is formed by evagination from the segmental duct. In the male it very early separates from the segmental duct to grow back free. Hoffmann was unable to determine whether or not the Wolffian duct remained connected for a short time with the pronephros after the formation of the Müllerian duct. Shortly after that event, the pronephros is certainly completely isolated. The ostium is at first quite shallow. The surrounding thickened peritoneal epithelium shares in its enlargement.

With the formation of the ostium, the degeneration of the pronephros begins. The first steps are the closure of the first nephrostome and the complete cutting off of the glomus from the body cavity by an outgrowth[1] from the radix mesenterii, which passes ventral to the glomus and fuses with the pronephric wall.

The second nephrostome remains after the complete disappearance of the rest of the pronephros. The Wolffian duct degenerates back almost to the mesonephros, even while the first nephrostome is still present. The glomus remains, in a modified condition, in half-grown animals, where it is entirely enclosed in the radix mesenterii.

In a short paper, which has not, so far as I know, been followed by any more extended account, Wilson (**'94**) gives the following description of the formation of the Müllerian duct in the Axolotl (Siredon pisciformis): "In a 25 mm. long larva one finds the coelomic epithelium of the portion of the body cavity that surrounds the glomerulus of the pronephros partially modified to form *a band of cylindric cells*, that runs close to the outer boundary of the space, in contact with the limit formed by the fusion of the lung and pronephros. *This band is a direct continuation backwards of the ciliated epithelium that forms the first pronephric nephrostome*, and where the lung frees itself from the pronephros the band spreads out laterally to form a plate of cylindric epithelium that extends far beyond the lateral boundary of the pronephros, but only to narrow again in the region of the second nephrostome, with the epithelium of which it fuses. Posterior to the second nephrostome the cylindric epithelium rapidly narrows to a thread of cells that lie outside the segmental duct. There can be no doubt as to the origin of these cells, for (1) the coelomic epithelium is markedly thickened and proliferating, and (2) the segmental duct is rounded and well defined, and shows no sign of budding off new cells or splitting. Sometimes the thickening is only one cell deep and three or four in breadth; sometimes it is several cells deep. It extends back at least as far as the mesonephros."

It will be seen that the condition here described is very similar to an early stage in Amblystoma. The thickening of the epithelium between the nephrostomes corresponds to what I have called the "thickened band," and that which "extends back at least as far as the mesonephros" is what I have termed the oviducal welt.

The author then goes on to say that in a larva twenty-seven millimetres in length the thickening in the pronephric region is very similar to that in the younger animal, although not so marked. Just behind the second nephrostome, however, there is a more marked proliferation of cells to form a mass [evidently corresponding to the "first evagination" after its caudal migration has begun] in the middle of which a rod appears. This rod is continued back for some distance, but is occasionally interrupted by merging with the cell mass, — or it may be represented by only a few heightened cells of the peritoneal epithelium. The cord is entirely distinct here, *as always* from the Wolffian duct.

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[1] Evidently this corresponds to the structure which I have designated as "shelf."

In still older specimens the rod becomes more marked, but shows the same irregularity as before; that is, the cord merges at times with the cells of the oviducal welt, or, if development has gone so far that a distinct tube is present, there are places where it is just appearing. In all cases the posterior end is continued as a simple epithelial thickening. During this caudad growth the anterior end of the thickening loses its connection with the degenerating nephrostomes. The thickening now extends from the region of the pronephros caudad and mediad almost to the mid-dorsal line. At this point, in a larva forty-five millimetres in length, the thickening begins to be raised into a ridge, and still farther back, grooved. Forty-eight sections behind the beginning of the fundament, the edges of the groove close over [probably the "second evagination" is here described] to form a rod, or, on one side of the body, a distinct tube for one or two sections, followed by the interrupted rod above described.

It is worthy of note that in the forty-five millimetre larva, the thickening begins to be raised to form a ridge at the point where it approaches the mid-dorsal line, the grooving beginning farther back, while in a specimen of fifty-one millimetres in length (which shows an advance in the differentiation of the Müllerian duct and hence really is older and not simply longer) the *grooving* begins at this point. Although the author does not call attention to the fact, there has evidently been a *progression cephalad* in the raising and infolding of the epithelium to form the duct.

The fact just alluded to makes me suspect that Wilson saw the ostium only after it had begun its forward migration, that is, some time after its first appearance. The free portion of the duct, unconnected with the ostium and present before that structure had appeared, and the isolated bits of duct might be explained as in my criticism of Fürbringer's description. It is needless to say that I consider Wilson in error in describing the posterior end of the developing duct as continuous with the peritoneal epithelium of the oviducal welt.

In Triton punctatus Gemmill ('97) describes the fundament of the Müllerian duct as first appearing while the pronephros is still completely functional and before its glomus has become enclosed in a glomerular cavity. At this stage the fundament appears as a thickening of the peritoneal epithelium lateral (morphologically ventral) to the second nephrostome. This thickening is reinforced by connective tissue, so that there is formed a pad which becomes a groove, probably by an evagination of the epithelium. The floor of the groove is continued as a solid cord extending caudad a short distance between the Wolffian duct and the peritoneum and *independent of both.* In slightly older larvae the peritoneal thickening also stretches caudad as a narrow welt (the oviducal welt) which indicates the path along which the Müllerian duct develops, but which *does not contribute cells to the growing duct.* The Müllerian duct develops from the cord described above. The groove is the fundament of the ostium. Immediately anterior to the mesonephros the cord fuses indistinguishably with the Wolffian duct, and in its further growth the posterior end is always represented by a thickening of the ventral side of that duct. As this growth proceeds, the proximal portion separates from the Wolffian duct, and receives a connective sheath of its own. The lumen of the duct appears as a prolongation of the lumen of the groove and progresses regularly caudad. Gemmill believes that the growth of the duct is brought about by a multiplication of its own cells with the addition of cells from the Wolffian duct. The ostium he considers as absolutely independent of the pronephric nephrostomes, and holds that the Müllerian duct cannot therefore be considered as representing in any way a duct of the pronephros. Aside from the lack of any mention of an anterior evagination and a cephalic displacement of the ostium, Gemmill's description is in close agreement with my own. The fact that I had not seen his paper until after my description of the oviducal development in Amblystoma, Rana, and Hyla was completed makes this agreement the more satisfactory to me.

Gymnophiona.

The fundament of the Müllerian duct is, according to Semon (**'92**), very simple in Ichthyophis, and entirely different from that of the higher Amphibia. It arises entirely independent of pronephros or its duct, from a thickening of the peritoneum. This thickening extends two or three segments anterior to the pronephros, and from it the ostium is formed — sometimes on a level with the pronephros, generally somewhat anterior to it — by the fusion of the edges of a groove which exists in the thickening. The lumen of the ostium is continued back for a short distance in the form of a tube which loses itself in the irregular mass of cells constituting the oviducal welt. The caudad prolongation of the duct, which is at first a solid cord, is formed by a rearrangement of the cells of the oviducal welt.

It will be seen that, contrary to what might have been expected in an animal which shows so many primitive characters in the urino-genital system, Ichthyophis has as yet thrown no light on the origin or development of the Müllerian duct in other forms.

Anura.

The conditions in Anura have been carefully studied by Hoffmann ('86) in the cases of Rana temporaria, R. esculenta, Bufo cinereus, by MacBride ('91, '92) in Rana, and by Gemmill ('97) in Rana temporaria and Pelobates fuscus.

According to the statement of Hoffmann, his was the first description of the development of the Müllerian duct in Anura. The first change preparatory to its development is a histological alteration of the pronephric duct between the pronephros and the mesonephros. This change consists in the transformation of its flat epithelial cells into those of a columnar shape and a reduction in the size of its lumen. The anterior end of the duct then separates from the degenerating pronephros and fuses with its peritoneal covering, — which is now also composed of cylindrical cells. A little anterior to the mesonephros, the segmental duct divides obliquely. Of the two portions thus formed, the posterior one, which ends blindly in front, retains its connection with the mesonephros and is the Wolffian duct. The anterior portion, connecting with the pronephric epithelium in front and ending blindly just lateral to the anterior end of the Wolffian duct, is the fundament of the Müllerian duct. The caudad growth of the Müllerian duct is independent of the Wolffian duct and is probably accomplished by the proliferation of a solid cord of cells from the neighboring thickened peritoneal epithelium, although of this Hoffmann is not certain.

Turning to the behavior of the anterior end of the Müllerian duct, its fusion with the peritoneal epithelium is converted into an opening. This is not, however, the ostium abdominale. From this opening a thickened band passes laterad and ventrad around the base of the lung and then turns caudad for some distance. By a folding of this band and a fusion of the lips thus formed the anterior opening is carried outward and downward, and then caudad. With the degeneration of the glomus, the duct moves mediad, and at the same time the edges of its cephalic opening begin to flatten out again to form a part of the coelomic epithelium. This retrogressive process is continued until the ventral, caudally directed portion of the duct has been obliterated, and the opening — now the ostium abdominale — occupies its adult position.

In the degeneration of the pronephros, the anterior nephrostome is the first to disappear, the second next, and, after some time, the third. It is in the vicinity of the surviving third nephrostome that the fusion of the pronephric duct with the peritoneum takes place.

I think there is no doubt that Hoffmann mistook the developing Müllerian duct for the anterior end of the pronephric duct. His description of the mode of formation of the duct anterior to the point where it first opens into the body cavity is in accord with what I found in Rana sylvatica, with the exception of the obliteration of a temporary, cephalic portion. The description of the degeneration of the pronephros is, of course, entirely different from my own results.

According to MacBride ('91, '92) there is no trace of the Müllerian duct in "the frog" until the tadpole has lost all its larval organs except the tail. The first trace of the duct is ventral to the only remaining nephrostome which, from its position, is probably the first. The fundament consists of a groove lined with columnar cells and open below. "This columnar epithelium is continued out over the surface of the pronephros and beyond it, as described by Hoffmann." The groove changes, posteriorly, into a canal which ends in a thickening of the peritoneum. Anteriorly, in later stages, the groove extends ventrad and becomes closed to form a canal "opening somewhat ventrally." Posteriorly, the thickening of the peritoneum, which constitutes the duct, runs back along a line of columnar epithelium which extends along the outer border of the mesonephros. MacBride calls the cord of cells which forms the duct a "thickening of the peritoneum" because it appears to be derived from the peritoneum, although he cannot be certain of the origin of its cells on account of the lymphoid tissue with which the outer boundary of the mesonephros is filled. In describing the cord he says "it appears [in cross-sections] as a nodule of deeply staining tissue, the outermost cells of which pass at the side into the ordinary epithelium." The duct is formed from this cord by the rearrangement of some of its cells in a stellate manner. Anterior to the mesonephros the cord "grows back with some regularity," but it appears posterior to the mesonephros long before it does along the side of that organ.[1] The lumen appears first anterior, then posterior, to the mesonephros. Posteriorly it appears in patches. MacBride saw no sign of a splitting of the pronephric duct as described by Hoffmann. He thinks it highly improbable that the portion just back of the pronephros takes any cells from the pronephric duct on account of the degenerate condition of the latter. Posteriorly, the growing cord comes nowhere in contact with the Wolffian duct.

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[1] One cannot help suspecting that MacBride confused the Müllerian duct and oviducal welt.

Gemmill ('**97**) describes the development of the Müllerian duct in Rana temporaria and Pelobates fuscus. The evagination or groove appears lateral [ventral] to the third nephrostome, and development proceeds much as in Triton punctatus (as described by him), with this important difference, — the growing duct comes nowhere in contact with the Wolffian duct. While its tip is too close to the oviducal welt to affirm that it takes no cells from that structure in Rana temporaria, in Pelobates cases were seen on both sides where the tip was undoubtedly free. The author's description of the cephalic displacement of the ostium agrees with my own for Rana sylvatica.

Amniota : Mammalia.

It is not my intention to discuss the many contradictory accounts which have been given of the origin and development of the Müllerian duct in Amniota. For a résumé of this subject the reader is referred to Burger ('**94**, '**94**^{a}). According to the researches of Kip ('**94**, '**94**^{a}), mammals seem to present the nearest approach in the development of the Müllerian duct to that found in Amblystoma, although from the account of its development in the chick given by Balfour and Sedgwick ('**79**), birds also show a close similarity. The parallel between Kip's ('**94**, '**94**^{a}) description and mine is so close that I shall recapitulate briefly his observations on *Insectivora* (Tupaia, Talpa, Erinaceus) and *Rodentia* (rabbit, mouse).

Tupaia : On the ventral surface of the anterior end of the mesonephros there is a thickening of the epithelium about 200 micra in length, which forms a plate of uniform thickness. At three successive points of this plate, cell proliferation takes place to form three massive cones, which then become pits opening into the coelom.[1] The inner ends of these (or rather of the first two, as the third generally degenerates) fuse, and the resulting cord grows back and applies itself to the blind anterior end of the Wolffian duct. The evaginations grow wider and fuse at their mouths to form a single large funnel. This union continuing inward, a single large duct is formed, which then grows in length cephalad by a fusion of the lips of the opening. In this manner the ostium is carried forward about 100 micra. In early stages, when the duct is represented by a cord which reaches back only about halfway along the mesonephros, the Wolffian duct contributes cells towards its formation. In older stages, when the cord has nearly reached the urino-genital sinus, it takes far fewer cells from the Wolffian duct and finally grows backward independent of that duct. Hence in Tupaia the Müllerian duct is derived from two entirely different sources :

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[1] The number of evaginations is variable. In one case but one was found; in another, four.

(1) The anterior end — that is, the ostium and the adjoining portion of the duct — is formed from the peritoneal epithelium.

(2) The remainder of the duct is formed largely from the Wolffian duct. Anteriorly, however, it takes a larger percentage of cells from the Wolffian duct than posteriorly.

Erinaceus: There are two evaginations. As the Wolffian duct reaches much farther cephalad than in Tupaia, the two cords from the evaginations reach it *before they have fused.* As a consequence the Wolffian duct must form two cords which fuse comparatively late. The anterior evagination often *loses its connection with the peritoneum,* so that the Müllerian duct appears branched anteriorly. The posterior portion of the duct takes *no* cells from the Wolffian duct.

Mouse: The "vertical" portion of the duct arises entirely independent of the ostium, for stages are found in which a considerable extent of the vertical portion is present unconnected with the ostium by the horizontal portion, which develops later. Kip suggests an explanation of this condition which consists in the assumption that a rudimentary evagination, homologous with one of those found in Erinaceus, gives rise to the vertical portion of the duct and then atrophies, leaving the anterior end of the duct to end free, — just as one branch occasionally does in Erinaceus. This free end, he suggests, then becomes connected with an ostium which arises later, this ostium not being homologous with any found in the Insectivora.[1]

In rodents the connection between Müllerian and Wolffian ducts is far less intimate than in Insectivora.

Kip states his belief that the development of the Müllerian duct has become independent of the Wolffian duct in reptiles, many birds, and possibly some mammals, by the extension cephalad of the method of formation seen in the posterior portion in insectivores. The ostia in mammals are homologous with the pronephric nephrostomes of elasmobranchs, but the ostia of insectivores cannot be homologous with the *same* nephrostomes as the ostia of rodents.

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[1] Kip found a rudimentary ostium in the rabbit posterior to the final ostium. The latter appears only after the degeneration of the rudimentary posterior one.

From the above abstract, it appears that a remarkable agreement exists between the development of the Müllerian duct in Insectivora and Amblystoma. In the rodents there would seem to be an additional process consisting in the formation of a second ostium, anterior to, and later than, the first. This condition might easily be derived from that in Amphibia by supposing that the lengthening of the duct, cephalad, was brought about not by a progressive infolding and fusion of the thickened band, *beginning at the ostium*, but in the following manner : The infolding and fusion begins at the *anterior* end of the band and progresses back to the ostium. The final result would be the same in both cases.

That the position of the evaginations in the Insectivora is as far back as the mesonephros could be explained by assuming that that portion of the tissue of the thickened band which gives rise to the evaginations migrates caudad *before* instead of after the proliferations have taken place.

For comparison I have briefly summarized below the observations of the various authors and my own.

Urodela.

Fürbringer: The Müllerian duct is formed from the Wolffian by a longitudinal splitting. The anterior end fuses *secondarily* with the thickened peritoneum to form the ostium. No cephalic migration of the ostial opening noted.

Hoffmann : The Müllerian duct is formed as described by Fürbringer, except that in the male only the more anterior portion of the duct arises from the Wolffian duct.

Wilson : The ostium and anterior end of the duct are formed by an evagination of the peritoneal epithelium. The rest of the duct arises from the peritoneal cells of the oviducal welt. He seems to have seen, without realizing the fact, two separate evaginations and a cephalic migration of the ostial opening.

Gemmill: The ostium and the anterior portions of the duct are from a peritoneal evagination. The remainder of the duct is developed partly from the Wolffian duct. No mention is made of a cephalic displacement of the ostial opening.

Hall: The ostium and the anterior end of the duct are formed from the peritoneal epithelium. This fundament had originally the shape of two separate evaginations. Only a small portion of the growing duct takes cells from the Wolffian duct. The remaining portion grows back independent of Wolffian duct and peritoneum. A cephalic migration of the ostial opening is brought about by the closure, from behind forward, of a thickened groove of peritoneal epithelium.

Gymnophiona.

Semon: The ostium and anterior end of the duct are formed from a peritoneal evagination, the remainder of the duct from cells of the oviducal welt.

Anura.

Hoffmann: The anterior portion of the Wolffian duct is converted bodily into the Müllerian duct. It separates from the pronephros and fuses with the thickened peritoneal epithelium to form the ostium. After dividing obliquely at the anterior end of the mesonephros, the anterior section grows back, probably with the aid of cells of the oviducal welt, to form the remainder of the Müllerian duct, the posterior section remaining as the Wolffian duct. A cephalic migration of the ostial opening takes place along a thickened groove of the peritoneal epithelium.

MacBride: The ostium and anterior end of the duct are formed from a peritoneal evagination, the remainder of the duct, at first in the form of discontinuous pieces, from cells of the oviducal welt. There exists a cephalic migration of the ostial opening similar to that described by Hoffmann.

Gemmill: The ostium is from a peritoneal evagination, and the entire duct is formed by a prolongation of the free end of this evagination. There is a cephalic displacement of the ostial opening as described by Hoffmann and MacBride.

Hall: My description is in agreement with that of Gemmill with the exception that evidences were seen of three original evaginations to form the ostial fundament.

Mammalia.

Kip: The ostium and the anterior end of the duct are from two or three separate peritoneal evaginations. A portion of the duct takes cells from the Wolffian duct. There is a cephalic displacement of the ostial opening similar to that described by me in Amblystoma and by various authors in Anura.

E. THEORETICAL CONSIDERATIONS.

Since Balfour and Sedgwick ('79) first suggested a homology between the evaginations in the chick which form the anterior end of the Müllerian duct, and the pronephros, there has been a tendency to derive the ostium in some way from the pronephric nephrostomes. Semon ('92) suggested that the eggs were originally emptied into the pronephric duct through canals homologous with the vasa efferentia of the testis, but that, on increasing in size, they fell directly into the body cavity and a pronephric tubule was specialized to transmit them to the pronephric duct, or to a duct derived from the pronephric by a splitting process.

In trying to trace the probable origin of the ostium and Müllerian duct, we are confronted by two very different conditions: In elasmobranchs the ostium is derived directly from the pronephric nephrostomes; in Amphibia the ostium *coexists* with the pronephric nephrostomes and is independent of them. It seems to me that these two conditions can be reconciled by supposing a diverging differentiation, somewhat after the following manner:

In the elasmobranchs, the large amount of yolk which serves for the nourishment of the young is, of course, a secondary acquirement. The ancestral form we may picture as having lived as a free larva, and as having possessed a pronephros which, in addition to functioning as an excretory organ, had to prepare itself for the function of carrying off, in the adult, the eggs set free in the body cavity. This was accomplished by the pronephric duct dividing[1] to form two potentially separate ducts, much as the hermaphroditic duct of the pulmonate gastropods is divided by a longitudinal infolding. The halves of the duct diverged from each other posteriorly and opened separately. Phylogenetically, this splitting and separation was carried cephalad until a stage of nearly complete independence was reached; then more or less of the posterior ends grew back independently of each other. With the acquisition of more and more yolk, the period at which the young fish began its independent existence (and more active metabolism) was retarded until finally the secretory function of the pronephros was superfluous, especially as the mesonephros arises early in elasmobranchs and reaches forward to the pronephric region. The same process — the accumulation of food material in the egg — necessitated an increased development of the duct which carried off the now enormous eggs, and the pronephros very early in ontogeny began its transformation into an ostium abdominale. This is accomplished in modern elasmobranchs, according to Rabl ('96), by a degeneration of some, and a fusion of the rest of the nephrostomes (Figs. *O*, *P*, p. 111). Thus we have in modern elasmobranchs a pronephros which consists of a number of serially arranged funnels whose tubules fuse to form the pronephric duct.[1] This duct divides longitudinally, one-half receiving the mesonephric excretions, the other remaining connected with the pronephric nephrostomes, or rather the single ostium derived from them.

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[1] That this division would separate the excretions of the pronephros from those of the mesonephros is of no consequence. The best explanation seems to me to be that of Van Wijhe ('89), who sees in it a means of preventing self-fertilization, — the early vertebrates having been, probably, hermaphroditic.

The ancestors of the Amphibia we can imagine as parting from those of the elasmobranchs at the stage when the pronephros still had a secretory function as well as a sexual one, but after the pronephric duct had acquired the double structure. With increase in cephalization, the pronephric tubules increased in number. Gradually there arose a division of labor, one set of tubules retaining the secretory function, the other set taking on the task of carrying off the eggs (Fig. *Q*). The loss in the number of secreting units thus brought about was compensated for by an increase in length and accompanying coiling of the anterior end of the pronephric duct to form the main bulk of the pronephros.[2] As the secreting action of the pronephros was retained during ontogenetic development until after the sexual or oviducal set of tubules had begun their transformation into an organ for carrying off the eggs, the secreting tubules retained their connection with the anterior end of the pronephric duct, while that part of the latter which split off to form the Müllerian duct was associated exclusively with the set of sexual tubules (Fig. *Q*). In Amblystoma (see Fig. *R*) we thus have the original pronephros repre-

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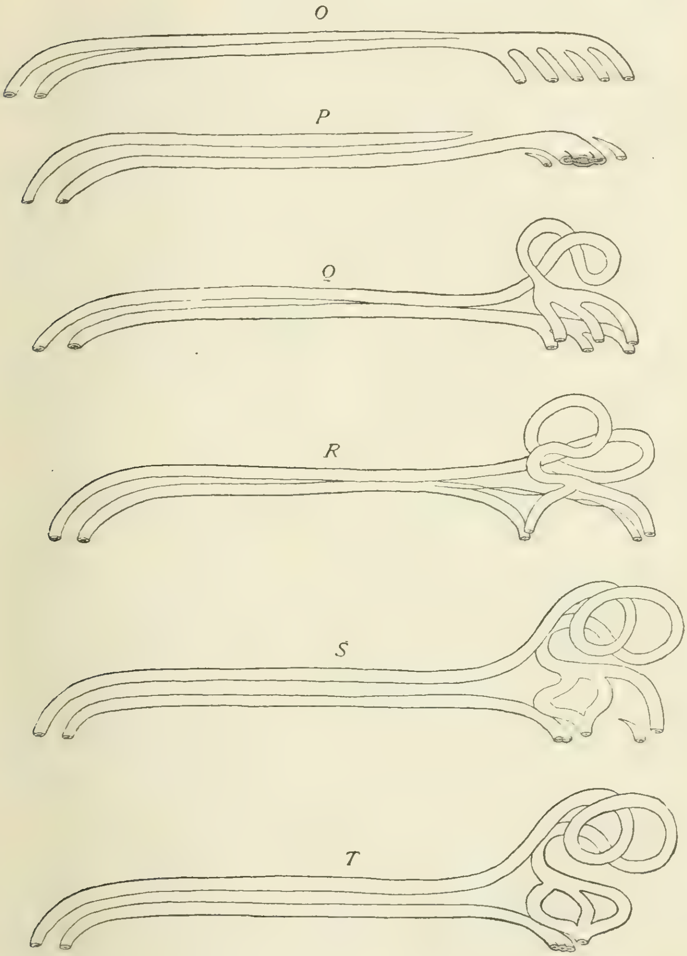
[1] Perhaps the tubules fuse *and join* the pronephric duct. The origin of the latter is still in dispute.

[2] According to Semon ('92), the anterior end of the pronephric duct in Ichthyophis, where the pronephric tubules number *twelve on each side*, does not form the massive coil characteristic of the higher Amphibia.



FIGURES *O–T*.

Six diagrammatic figures representing the relation of the Müllerian evaginations to the nephrostomes, the Müllerian duct to the Wolffian, etc. The Müllerian evaginations are somewhat below the nephrostomes in each case and the Müllerian duct is below the Wolffian.

Figures *O* and *P* represent two stages in elasmobranchs; Figure *Q*, a hypothetical, primitive condition in Amphibia; Figure *R* shows the condition in Amblystoma; Figure *S*, that in Hyla; and Figure *T*, that in Rana.

sented by (1) two dorsal tubules (the secretory portion), whose fused ends are continued backward as the pronephric duct, and (2), ventral to these, two tubules (the sexual portion) arising from Müllerian evaginations, whose caudal ends join the pronephric duct some distance behind its coiled, anterior end. It would seem that in the ancestors of the Urodela and Anura there was originally one pronephric tubule for each of several segments and that the number was increased by the addition of a tubule in each of the segments already containing one (Figs. *R*, *S*, and *T*). These additional tubules lost their secretory function and took on a sexual one. The exact position of the sexual tubules in the somite is not significant. It seems probable that they arose ventral to the secreting set, for that is their position in Amblystoma and Rana sylvatica. In Hyla versicolor, it is true, they arise dorsally, but this position may have come about secondarily, as is suggested by the fact that in one case a Müllerian evagination was seen which was *ventral* to the nephrostome.

Such a duplication of tubules as postulated above seems to take place in Amphiuma, where, according to Field ('94), three somites on each side possess two tubules each, one dorsal and one ventral, making six in all. Whether one of these sets, the dorsal or the ventral, gives rise to the Müllerian duct or not, we do not know, as the development of the duct has not been studied. Such a result would not, however, be essential to a confirmation of the theory here set forth, as it is quite possible that in groups other than the Urodela and Anura the Müllerian evaginations have been derived from a third set of pronephric tubules, or from tubules of the posterior or anterior end of the single original series. In the latter case there would be no necessary correspondence in number between Müllerian evaginations and secretory tubules.

In Amblystoma (and probably all other forms above the elasmobranchs) the appearance of the sexual tubules is delayed, so that they arise much later than the secretory set. Their distal ends fuse with each other (to form the anterior end of the Müllerian duct) before they reach the Wolffian duct in all the cases that I have examined; but as the point of fusion is quite variable, they may sometimes reach the duct separately.[1] The original formation of the Müllerian duct in the remote ancestors of the Amphibia by a splitting of the Wolffian duct is indicated by the fact that the latter contributes cells to the growing Müllerian duct in the region where the two come in contact. Behind this point the Müllerian duct grows back independently. This independent growth probably represents an extension cephalad of the process whereby the posterior end of the Müllerian duct reached a separate external opening. As suggested by Kip ('94, '94[a]), one can imagine this process as phylogenetically advancing cephalad until the posterior prolongation of the ostial evagination reaches the cloaca without establishing any connection with the Wolffian duct. This stage seems, indeed, to have been reached in the Anura.[1] A connection between the two ducts has been claimed, however, for members of every group of the vertebrates which possess a Müllerian duct, with the exception of the Reptilia. It may be noted, also, that the reptiles are the only remaining group (since my observations on the Amphibia now exclude that group) in which we have not some evidence that there is more than one evagination as forerunner of the ostium abdominale.[2]

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[1] Kip ('94, '94^a) notes such cases in mammals (see page 106).

F. RECAPITULATION OF THE DEVELOPMENT OF THE MÜLLERIAN DUCT.

Amblystoma.

The first trace of the fundament of the Müllerian duct appears, some time before the degeneration of the gills and pronephros, in the shape of a thickening of the peritoneal epithelium beneath the first nephrostome. This thickening forms a band of crowded, cylindrical cells, which extends ventrad from the nephrostrome, but is soon forced to turn caudad by the growth of a "shelf" of tissue which extends horizontally across that portion of the body cavity which is dorsal to the fusion of the lung with the lateral body-wall. This shelf thus divides the dorsal portion of the body cavity into an upper chamber, the glomerular cavity, and a lower chamber, the sub-glomerular cavity.

Soon the thickened band extends from the first nephrostome back along the dorsal surface of the shelf, then ventrad around its posterior edge and forward again on its ventral surface. As it passes back beneath the second nephrostome it is joined by a similar band from that organ.

Immediately ventral to the first nephrostome there appears a local accentuation of the thickening to form a small, thick disk. A similar one then appears beneath the second nephrostome. A depression in the surface of each of these disks results in the formation of an open pit, the wall of which extends into the substance of the pronephros.

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[1] Figures *S* and *T* (page 111) represent the conditions in Hyla and Rana, — a total independence of Müllerian and Wolffian ducts. They also show, when compared with Figure *Q*, the modifications undergone by nephrostomes and Müllerian evaginations.

[2] Burger ('94, '94[a]) describes the duck as having as many as fifteen.

The portion of the glomerular cavity surrounding the mouth of the anterior of the two pits and the neighboring nephrostome becomes partially closed off to form what I term the nephrostomal cavity.

From the distal (deep) end of the first pit, or Müllerian evagination, two ducts take their origin, one of which, the *precoelomic duct*, extends cephalad and later degenerates completely. The other, which I have called the first duct, ignoring the precoelomic duct, extends caudad past the second evagination and there fuses with a similar (second) duct from that evagination. That portion of the first duct which lies anterior to the union with the second duct degenerates.

In the mean time both evaginations have migrated caudad. This movement withdraws the first evagination from the nephrostomal cavity (which closes and with its associated nephrostome disappears) and brings the second to lie behind the posterior limit of the shelf, and hence out of the glomerular cavity. The thickened epithelial band is consequently no longer forced to make a detour to reach the second evagination; it extends from the anterior end of the sub-glomerular cavity, back along the ventral surface of the shelf (or along the adjoining lateral wall of the body), and is continuous through the second evagination, with the duct leading caudad from the latter.

The band from the first to the second evagination disappears. The first evagination also degenerates completely.

That part of the Müllerian duct which is immediately posterior to the second evagination is composed of the duct of that evagination *plus* a portion of the duct of the anterior evagination. After the fusion of these two components to form a single duct, the latter grows back near the Wolffian duct, fuses with it for a variable, but never great distance, and then grows backward free. Later, the fused portion again separates from the Wolffian duct. That the Wolffian duct contributes cells helping to form the Müllerian duct in this region, seems almost beyond question. The greater part of the duct, however, — that is, throughout the entire extent of the mesonephros, — grows back independent of the Wolffian duct.

The Müllerian duct increases in length cephalad by utilizing the thickened band which extends anterior to the second evagination, that being the only one which persists. This is accomplished by a folding of the band into a groove, beginning at the evagination and progressing cephalad. The fusion of the edges of the groove carries the opening of the duct cephalad and ventrad to its position in the adult.

Rana sylvatica.

Preceding the degeneration of the pronephros, the three nephrostomes move closer and closer together and finally open into a common outpocketing of the coelom, which forms a narrow, ciliated canal closely resembling the primary nephrostomes. This "common nephrostome" is comparable to the "nephrostomal cavity" in Amblystoma in that, by its closure, it severs the connection of the nephrostomes with the coelom, just as the closure of the "nephrostomal cavity" shuts off the first nephrostome.

As a result of the migration of the nephrostomes to a common point, the tissue of the peritoneum which was immediately ventral to each is represented by tissue ventral to the common nephrostome. It is suggested that this tissue contains the fundaments of the three Müllerian evaginations which should theoretically be present if there is a correlation between the number of evaginations and nephrostomes, as the condition in Amblystoma suggests. As a consequence of the condensation of the tissue of the three fundaments, the latter are generally represented by an irregular mass of proliferated cells. This mass is, however, elongated in the direction of the long axis of the body and usually shows two or three distinct regions of heightened proliferation, or may even show more than one distinct evagination. A fundament is figured in which there are two distinct evaginations and a proliferated cone which may represent a third. An additional proof that there was originally a Müllerian evagination associated with each of the three nephrostomes is seen in the fact that in one larva, in which the fusion of the nephrostomes had been retarded, there is one distinct evagination beneath the common opening of the first and second nephrostomes and a thickened disk, probably representing another, beneath the third nephrostome, which opens at some distance behind the other two.

In the case just mentioned, there are two thickened bands which fuse and extend cephalad and ventrad. In normal cases there is a single band. As the shelf in Rana sylvatica is a very transitory structure of small extent, the curving of the thickened band in passing forward to the sub-glomerular cavity, which is such a prominent feature in Amblystoma, is barely represented. Soon after the appearance of the evaginations, the band forms a conspicuous groove or fold, open below, so that cross-sections of it have the form of an inverted V. The dorso-median wall of the groove later becomes continuous with the dorso-median lip of the single remaining opening of the Müllerian duct, and the latter is carried cephalad and ventrad by the progressive fusion of the edges of the groove.

The portion behind the Müllerian evagination is formed from a prolongation of its distal end. It was found impossible to decide whether the evagination was a single one, remaining after the degeneration of others, or a compound one formed by fusion. That the Wolffian duct contributes cells to the growing Müllerian duct seems improbable from the fact that, in the only region where the relationship between the two ducts cannot be clearly distinguished, the Wolffian duct is very degenerate. Posterior to that point the two ducts seem to be wholly independent of each other.

Hyla versicolor.

The phenomena exhibited in the degeneration of the pronephros of Hyla differ from those in Rana in one important particular, — the fusion of only the two posterior nephrostomes, instead of all three, to form a "common nephrostome." In consequence of this peculiarity, the evagination associated with the first nephrostome is allowed as free a development as the anterior evagination in Amblystoma. The fusion of the two posterior nephrostomes, however, brings about a crowding of tissue similar to that in Rana, and as a consequence but one evagination appears. This posterior evagination, from which the Müllerian duct develops, may represent the third (supposing one originally associated with each nephrostome), or it may represent a fusion of the second and third. Efforts to resolve it into two were unsuccessful in normal cases. In the abnormal case in which the two posterior nephrostomes remained separate, there was strong evidence of an evagination associated with the second in addition to those unquestionable ones associated with the first and third.

In regard to the establishment of the adult ostium, Hyla resembles Amblystoma far more closely than it does Rana. The cephalic displacement of the opening of the young Müllerian duct takes place along a trough-like groove which extends from the posterior evagination to the anterior, and is there continuous with a thickened band which extends cephalad and ventrad toward the point where the adult ostium is situated. The process of displacement was observed only in its initial stages, but at those stages it is accomplished by a progressive fusion of the lips of the groove in a manner similar to that seen in Amblystoma, and there can be little doubt that the remainder of the process is essentially as in that form.

To account for the two very different modes of origin of the Müllerian ducts found in elasmobranchs on the one hand and in the Amphibia (and probably the Amniota) on the other, it is suggested that the Müllerian evaginations in the Amphibia studied by me represent a ventral set of pronephric tubules comparable if not homologous with the ventral set in Amphiuma. This ventral set, which originally possessed a secretory function, has become, like the pronephric tubules of elasmobranchs, specialized to subserve a sexual function. Instead of opening into the pronephric duct, they form a duct of their own which, by fusing with the pronephric duct for a short distance in Amblystoma and many Amniota, still betrays the fact that it had its origin in a splitting of that duct.

New Haven, May, 1902.

Addendum.

Too late to be incorporated in the body of the text, there came to hand an extremely important paper by Brauer (:**02**). I shall refer to such parts only as have a direct bearing on points treated in my own paper. His material consisted of specimens of *Hypogeophis rostratus*, belonging to the Gymnophiona.

The mesonephros is very long, extending throughout seventy-six segments, — from the 24th to the 100th. Two greater divisions may be distinguished, that comprising the region of segments 24 to 29, and that including segments 30 to 100. In the former division the mesonephric units remain rudimentary and finally degenerate; in the latter both primary and secondary are formed, the primary reaching a functional stage. This posterior division also shows a differentiation into two regions; in segments 50 to 100, the secondary tubules become fully developed; in segments 30 to 50 they degenerate. The degeneration of the secondary units brings about a strictly metameric arrangement of the tubules in this region of the adult kidney, for the entire organ has from the beginning a metameric structure to the extent that there is but one *primary* unit in each segment.

As the units of the last ten mesonephric segments (somites 90 to 100) are peculiarly modified, typical development is present in only forty segments (somites 50 to 90). The development of a mesonephric unit in this region is as follows: the somites are constructed essentially as in Amblystoma, their lumina being, however, larger. The ventral (lateral) portion of the somite, corresponding to what I have called the mesomer, is in the form of a tube whose lumen sets the lumen of the rest of the somite (the epimer) in communication with the general body cavity. This ventral, tubular portion of the somite becomes *directly transformed into a mesonephric blastula.* In the more posterior segments it becomes cut off from the lateral mesoderm, a slight widening of its lumen takes place (a process designated by Brauer as the formation of the *nephrostome*), and it becomes constricted from the epimer. In the median portion of the typical region, the widening of the lumen of the mesomer takes place *before* that structure loses its connection with the lateral plates. In either case the result is the transformation of the lower portion of the somite (the mesomer) into a single rounded mesonephric blastula, which is independent of all other organs. Very significant is the fact that in the more anterior portion of the typical region, certain of the mesomers *never lose their connection with the lateral mesoderm*, so that their splanchnoderm and somatoderm remain continuous with the corresponding layers of the lateral mesoderm and their lumina continuous with the general body cavity. The outer tubule and nephrostome of a primary unit may thus be formed, in certain cases, by a simple transformation of structures present from the very beginning of somite differentiation. This, as has already been noted, is typically the case in elasmobranchs. It will be remembered that there was evidence of a similar retention of continuity between mesomer and lateral mesoderm in the more anterior portion of the mesonephros of Amblystoma. In that form, however, a continuous lumen was not recognizable.

The formation of a mesonephric blastula in Hypogeophis is thus similar to that in Amblystoma with the important exception that in the former genus the entire mesomer is transformed directly into a single primary unit, its lumen being not even temporarily obliterated.

The differentiation of the primary blastula is much as in Amblystoma. As in that form, the inner tubule arises as an *evagination of the lateral wall* of the blastula and is forced dorsad by the proximity of the Wolffian duct. If the peritoneal connection has not been retained, it is established by means of a nephrostomal evagination appearing at the ventrolateral angle of the blastula. The *secondary blastula* is then formed by an outbudding of the posterior, median portion of the blastula. It will be seen that the secondary blastula arises from the primary at a later period than in Amblystoma. The secondary blastula resembles that of Rana sylvatica in that it may retain its connection with the primary by means of a cord of cells. Where this occurs, it is seen to be attached to the primary Malpighian body at a point similar to that of the attachment in the case of Rana.

Brauer lays stress on the fact, likewise emphasized in my introduction, that the inner tubule *grows out* from the *lateral* side of the blastula and is comparable with the pronephric tubule, while the Malpighian body (Bowman's capsule) is formed from the blastula by a direct transformation.

The history of the secondary unit differs somewhat from that in Amblystoma. After it is cut off from the primary, it lies close against the Wolffian duct, posterior to the opening of the main tubule of the primary unit. Where it touches the duct, a dark-staining evagination of that organ grows out, pushing the blastula before it. A long, tubular evagination of the Wolffian duct is thus formed into which the secondary tubule later opens. Differentiation of the secondary blastula is very similar to that of the primary. It develops in turn an evagination to form the inner tubule, one to form the outer tubule and one which becomes the tertiary unit. The tertiary blastula develops an inner tubule which meets and empties into a tubular outgrowth from the previous evagination of the Wolffian duct.

As many as eight units may be present in a single somite. Brauer is not certain that any beyond the quaternary form outer tubules and nephrostomes, but they may possibly connect with the body cavity in the manner in which the most posterior secondary units occasionally do, which is as follows. In Segments 90 to 100 the secondary outer tubule, instead of joining the peritoneum directly, sometimes joins the outer tubule of the primary unit, close to its nephrostome.

In the posterior segments a much more common condition than the one just described is the failure of both primary and secondary tubules to connect with the peritoneum. Instead, the outer tubule of the primary unit, avoiding the peritoneum, turns aside, meets and fuses with the outer tubule of the secondary unit, thus putting the two Malpighian bodies into connection with each other.

The most important, and, to me, satisfactory result of Brauer's work, the establishment of a close similarity between the mode of development of the pronephric and mesonephric units, I may not discuss, as it would carry us beyond the province of my paper. There remains, however, the *development of the Müllerian duct.* The first process in the formation of the Müllerian duct is a thickening of the lateral wall of the anterior half of the pronephros. On this thickened plate two longitudinal ridges arise. The free edge of the upper one folds ventrad, that of the lower, dorsad. On following the ridges caudad they are seen to form a tube by the coalescence of the folded edges. This tube, the Müllerian duct, soon ends blindly in close proximity to the peritoneum. The latter does not, however, contribute cells to the growing duct. The ostium seems thus to be formed entirely from one Müllerian evagination, but from both the description and figures of Brauer (compare especially Fig. 145, Taf. 9) there is no doubt in my mind that several other evaginations in a degenerate condition are present posterior to the chief one. Such an interpretation of the structures in question occurred to Brauer himself, for he says in describing them (page 140): "Weiter caudalwärts" (referring to the permanent ostial evagination) "erkennt man zwar noch erhötes Epithel, auch, wenn auch unregelmässig, scheinbare Anfänge von einer Faltenbildung, so dass der Anschein erweckt wird, als ob das Peritonealepithel in ähnlicher Weise wie zur Bildung der Tube sich rinnenförmig einsenke." One other similarity to the development of the duct in Amblystoma consists in a caudad transference of the ostium from its original position on the anterior half of the pronephros to one at the anterior end of the mesonephros. In Amblystoma the caudad migration is not so great and is seen in the Müllerian evagination, not in the adult ostium.

June, 1902.

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EXPLANATION OF PLATES.

All of the figures of sections were drawn with an Abbé camera and represent their anterior faces, so that what appears at the left in the figures is really at the right in the animal.

ABBREVIATIONS.

ao.	Aorta.
cav. glm.	Glomerular cavity.
cav. nph'stm.	Nephrostomal cavity.
cav. sb'glm.	Subglomerular cavity.
cl. g.	Germ cell.
coel.	Coelome.
coel. d.	Dorsal division of body cavity.
coel. so.	Coelome of somite.
coel. v.	Ventral division of body cavity.
cps. Bow. ex.	Outer wall of Bowman's capsule.
cps. Bow. i.	Inner wall of Bowman's capsule, which forms the glomerular covering.
crs. g.	Germinal ridge.
crs. o'dt.	Oviducal welt.
crv. cp. Mpg.	Neck of Malpighian body.
dt. 1; dt. 2	Ducts from the first and second Müllerian evaginations.
dt. Muel.	Müllerian duct.
dt. precoel.	Precoelomic duct.
dt. Wf.	Wolffian duct.
ec'drm.	Ectoderm.
e'coel.	Epicoelome.
e'mer.	Epimer.
evg. 1, 2, 3	First, second, and third Müllerian evaginations.
fnd. ms'nph.	Fundament of mesonephros.
glm.	Glomus or glomerulus.
hp.	Liver.
marg. p. tab.	Posterior margin of the "shelf."
ms'drm. l.	Lateral mesoderm.
ms'ent.	Mesentery.
ms'mer.	Mesomer.
my'cm.	Myocomma.
my'tm.	Myotome.
nph'stm.	Nephrostome.
nph'tm.	Nephrotome.
nt'cd.	Notochord.
pi'cr.	Pericardium.
pn.	Lung.
pn. + par.	Fusion of lung with the lateral body-wall.
scl'tm.	Sclerotome.
so'drm. l.	Somatoderm of the lateral mesoderm.
so'drm. t.	Transverse part of the somatic wall of the somite.
so. v–l.	Ventro-lateral angle of somite.
spl'drm. l.	Splanchnoderm of the lateral mesoderm.
spl'drm. t.	Transverse part of the splanchnic wall of somite.
sul.	Groove.
tab.	"Shelf."

tae. e'th. α	Thickened epithelial band from the anterior evagination.
tae. e'th. β	Thickened epithelial band from the posterior evagination.
tae. e'th. γ *tae. e'th.* γ'	The band posterior to the fusion of portions α and β. That which is dorsal to the shelf is designated by γ, that ventral by γ'.
tbl. ms'nph.	Mesonephric (inner) tubule.
tbl. nph'stm.	Nephrostomal (outer) tubule.
tbl. pr'nph. 1, 2, 3	First, second, and third pronephric tubules.
trn. clg.	Collecting trunk.
trt. in.	Alimentary tract.
va. sng.	Blood-vessel.
vst. nph.	Nephrostomal vestibule or common nephrostome.

PLATE 1.

All figures are of cross-sections and are of *Amblystoma* unless otherwise indicated.

FIG. 1. Stage I. A section through the middle of the seventh somite of the right side of the body. × 100.

FIG. 2. Stage IV. Through the middle of the seventh somite of the left side.

FIG. 3. Stage II. Through the middle of the sixth somite of the right side. × 100.

FIG. 4. *Rana.* Through the ninth (lower) and tenth (upper) somites of the left side. × 280.

FIG. 5. Stage II. Through the middle of a somite of the right side, showing the germ cells in both layers of the lateral mesoderm. × 250.

FIG. 6. *Rana.* Through the anterior half of the sixth somite of the left side of a larva 3.25 mm. long. × 280.

FIGS. 7, 8, 9. Stage V. Three sections through somites of the right side of the body. × 335.

FIG. 7. Cuts somites 11 and 12.

FIG. 8. Passes through the middle of somite 13.

FIG. 9. Passes through the posterior end of somite 13.

FIGS. 10, 11, 12. Stage VI. × 335.

Fig. 10 represents a section through the thirteenth somite of the right side.

Figs. 11, 12 represent sections through the twelfth somite of the left side.

HALL—MESONEPHROS IN AMPHIBIA. PLATE I

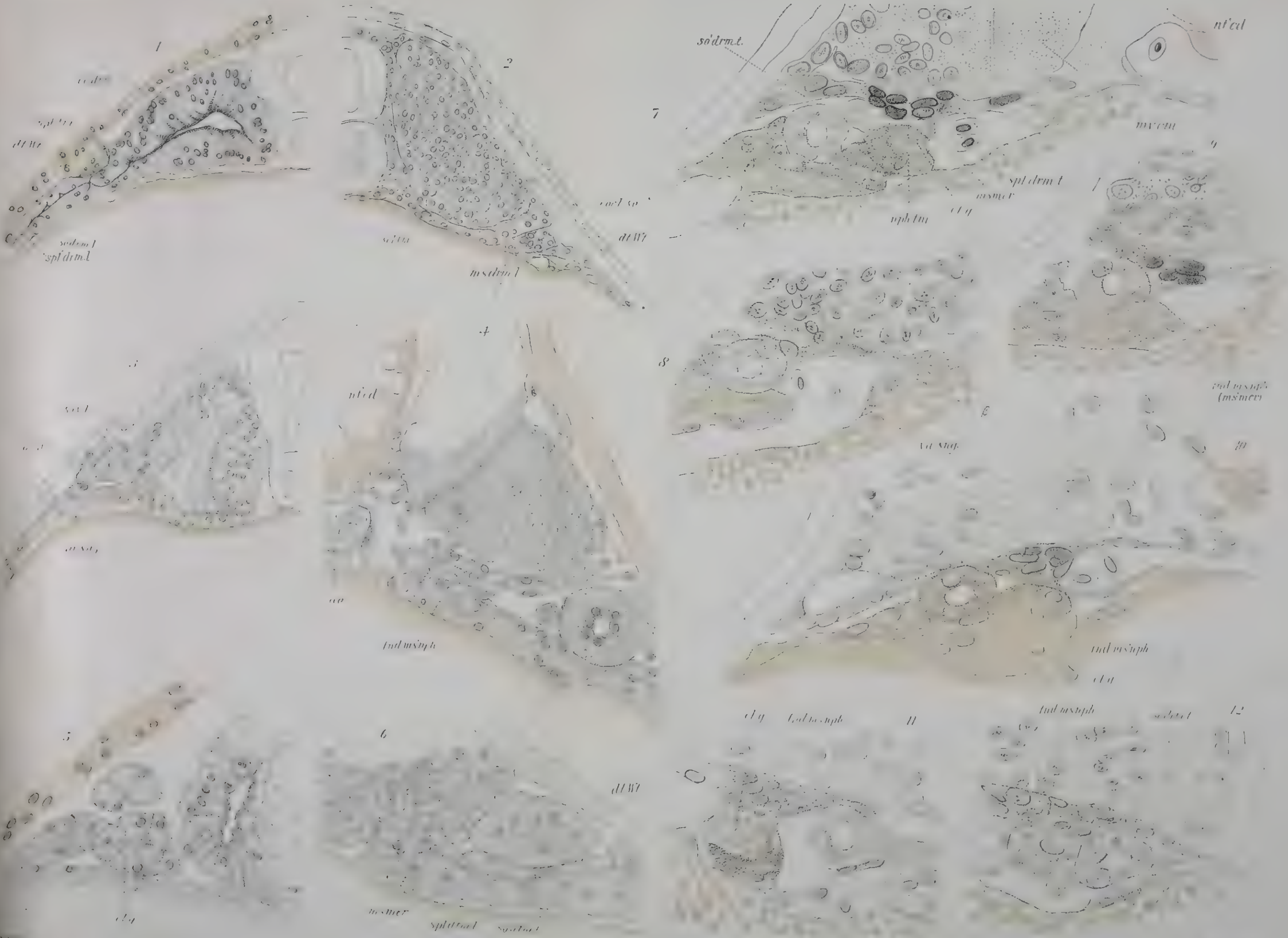
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PLATE 2.

All figures are of cross-sections and are of *Amblystoma* unless otherwise indicated.

Fig. 13. Stage III. Through the middle of somite 13 of the right side. × 335.

Fig. 14. *Rana.* Through the anterior half of the seventh somite of the right side of a larva 4.5 mm. long. × 280.

Fig. 15. *Rana.* Through the last mesonephric blastula of the left side of a larva 8.5 mm. long. × 410.

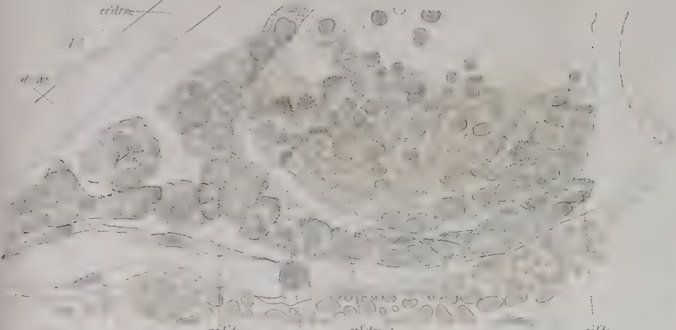
Fig. 16. *Rana.* Through the eighth somite of the right side. × 380.

Fig. 17. Stage VII. Through the posterior part of somite 11 of the right side. × 560.

Fig. 18. *Rana.* One of the sections from which Figure 102 was constructed. The larva measured 11.5 mm. in length. × 410.

Fig. 19. Stage VIII. Through the eleventh somites of both sides of the body, showing two mesonephric blastulae. × 305.

Figs. 20–23. Sections of four mesonephric units. All are from the posterior half of the sixteenth somite. That of Figure 22 is from the right side, the others from the left side of the body. The blastula of Figure 20 is in reality smaller than that of Figure 21, Figure 20 being magnified 500 diameters, while Figures 21, 22, 23 are magnified only 340 diameters.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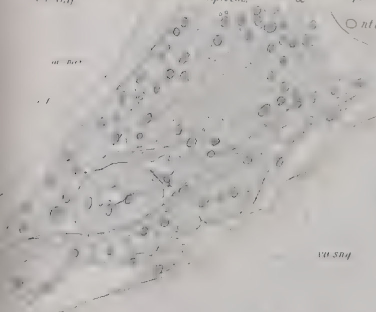
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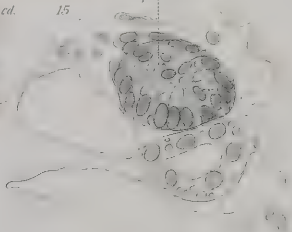
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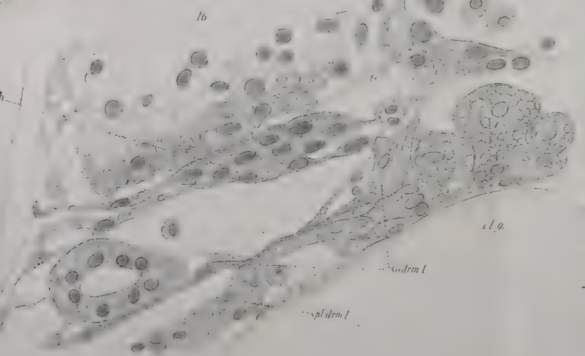
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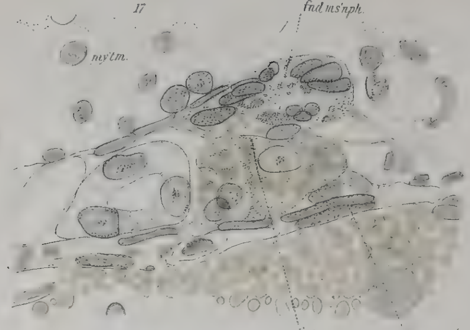
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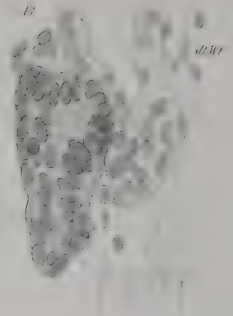
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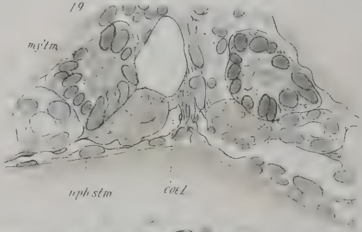
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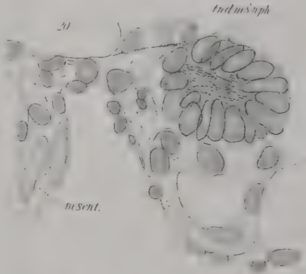
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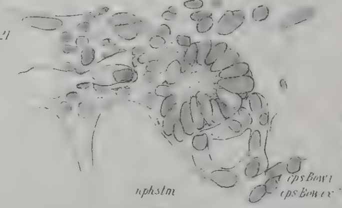
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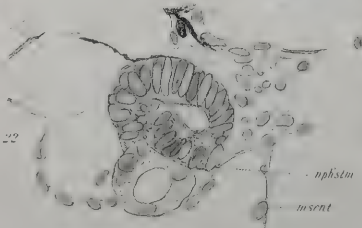
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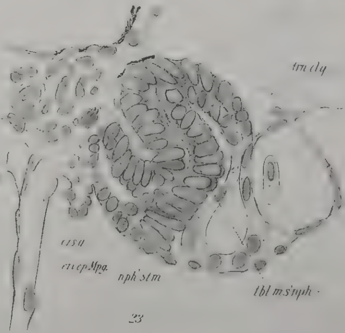
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PLATE 3.

All figures, except 30, 34, and 35, are of cross-sections, and all are of *Amblystoma* unless otherwise indicated.

Fig. 24. Through somite 18 of the right side of a young adult about 60 mm. long. × 75.

Fig. 25. Through a primary and a secondary mesonephric unit from the left side of a larva 25 mm. long. × 188.

Fig. 26. Through a primary and secondary mesonephric blastula from the eighteenth somite of the right side of a larva 24 mm. long. × 350.

Fig. 27. Section of a primary, a secondary, and a tertiary unit from somite 18 of the right side of a larva 25 mm. long. × 195.

Fig. 28. *Rana.* Section of a mesonephric unit from the right side of the body. The same unit is reconstructed in Figure 30, a dotted line in this figure indicating the level of the section shown in Figure 28. × 410.

Fig. 29. *Rana.* A section showing a tertiary blastula still connected with the Malpighian body of a secondary unit, from the right side of the body. × 410.

Fig. 30. *Rana.* A dorso-median view of a wax reconstruction of four mesonephric units and the Wolffian duct of the right side of the body. For a drawing of a cross-section of the most posterior of the four units, see Figure 28.

Fig. 31. Larva I. A cross-section through the first pronephric nephrostome of the left side of a larva 23 mm. long. × 36.

Fig. 32. Larva II. Section passing immediately posterior to the first pronephric nephrostome of the right side of a larva 25 mm. long. × 48.

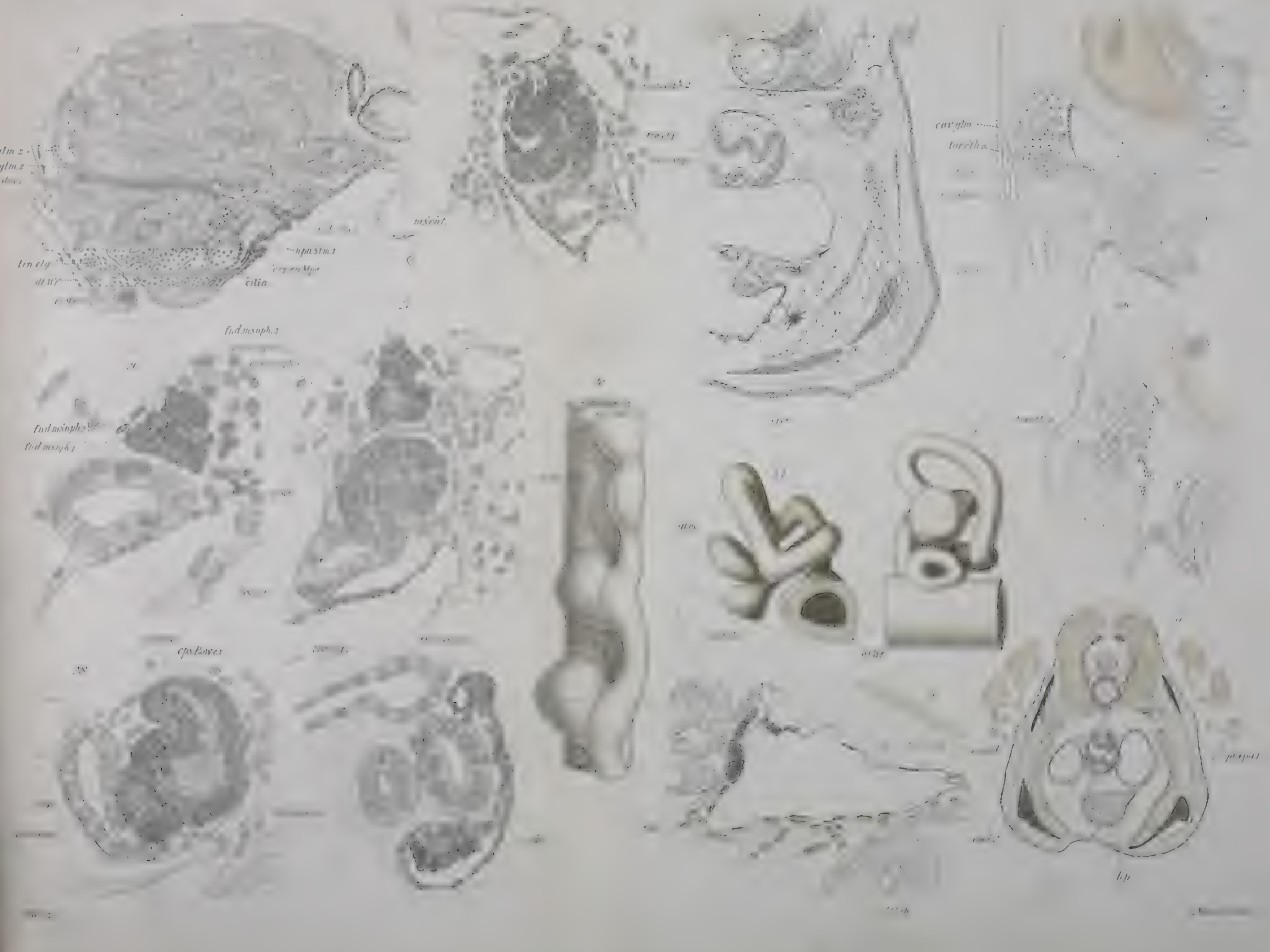
Fig. 33. The third section behind that shown in Figure 32. × 48.

Figs. 34, 35. Two drawings of a wax reconstruction of a mesonephric tubule of left side of the body of a larva 16 mm. long. Figure 34 is the anterior aspect; Figure 35, the ventro-median. × 120.

Fig. 36. Larva III. Through the first pronephric nephrostome of the right side of a larva 21.5 mm. long, showing the fundament of the anterior Müllerian evagination. × 147. Note. — By oversight of the lithographer the nuclei of the tubules are left unshaded.

Fig. 37. Through the second nephrostome of the left side of the larva shown in Figure 31. × 16.

HALL—MESONEPHROS IN AMPHIBIA.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fnd msnph.1
cps Bowe
car glm
lp

PLATE 4.

All figures are from cross-sections of *Amblystoma*.

Fig. 38. Larva IV. Through the second nephrostome of the left side of a larva 44 mm. long. × 118.

Fig. 39. The fourth section posterior to that shown in Figure 38. It passes through the nephrostomal cavity. × 118.

Fig. 40. Larva V. Through the second nephrostome and the second Müllerian evagination of the right side of a larva 41 mm. long. × 100.

Fig. 41. Through the nephrostomal cavity and the fundament of the first Müllerian evagination of the left side of the larva represented in Figure 40. × 118.

Fig. 42. Larva VI. Through the anterior evagination of the right side of a larva 40 mm. long. × 200.

Fig. 43. Third section posterior to that of Figure 42. × 200.

Fig. 44. Larva VI *b*. Through the second nephrostome and the second (posterior) evagination of the left side of a larva 42 mm. long. × 200.

Fig. 45. Larva XII. Through the degenerate pronephroi of a larva 55 mm. long, in which the gill-slits have closed. It shows, on each side, the glomerular cavity and the epithelial band some distance anterior to the ostium. × 88.

Fig. 46. A section posterior to that of Figure 45, showing the opening of the right glomerular cavity into the coelome. × 118.

Fig. 47. Through the ostium of the left side of the larva shown in Figures 45 and 46. × 118.

Fig. 48. Larva VII. Through the right pronephros of a larva 35 mm. long, showing the precoelomic duct. × 200.

Fig. 49. Larva XI. Through the first Müllerian evagination (retained to a very late stage) of the left side of a larva 49 mm. long. × 156.

Fig. 50. Larva IX. Section showing the process of fusion of "duct 1" and "duct 2" of the left side of a larva 50 mm. long. × 335.

Fig. 51. A section anterior to that of Figure 50, passing through the second Müllerian evagination of the left side. × 156.

Fig. 52. Through the opposite side of the same larva as that represented by Figure 49, showing a remnant of "duct 1" and the epithelial band. × 130.

Fig. 53. The sixth section behind that drawn in Figure 44, showing the dwindling end of "duct 2" and the oviducal welt. × 250.

mytm
tacëthy'
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trtin.
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crg1
cavglm
dtW
dtWf
tacëth a
dtWf
dt1
crg2
sul.
tacëthy'
tacëth a

PLATE 5.

All figures are of cross-sections, 54–64 of *Amblystoma;* 65–67 of *Rana.*

FIGS. 54–64. Larva X. Eleven figures showing the relation between the Müllerian and Wolffian ducts. Figures 54 to 63 represent consecutive sections, of which 54 is the most anterior; Figure 64 a section considerably posterior to that of Figure 63. × 420.

FIG. 65. Through the common nephrostome of the right side of a larva 25 mm. long. × 56.

FIG. 66. Through the common opening of the first and second nephrostomes of the right side of a larva 37 mm. long, showing the first (first + second?) Müllerian evaginations. × 195. NOTE. — By an oversight the nuclei of the tubules in Figs. 66, 67, are left unshaded.

FIG. 67. A section immediately anterior to the third nephrostome of the right side of the larva of Figure 66, showing the fundament of the second (third?) Müllerian evagination. × 195.

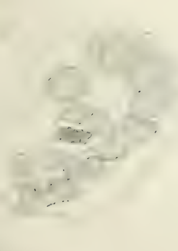

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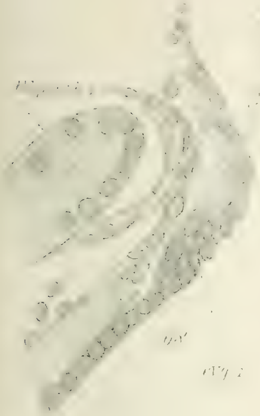
PLATE 6.

Cross-sections of *Rana*.

FIGS. 68–77. Ten consecutive sections through the Müllerian evaginations of the right side of a larva 34 mm. long. The fundament is seen to be resolvable into an anterior, questionable evagination (Fig. 68) and two distinct ones (Figs. 74, 76). Figure 77 is on Plate 7. × 315.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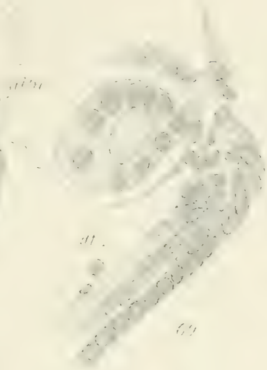
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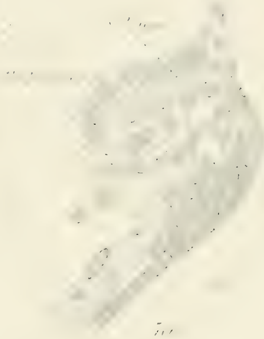
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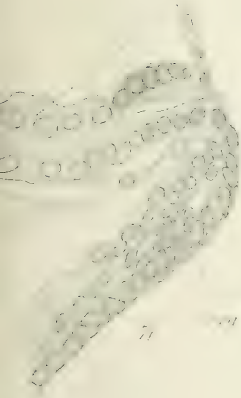

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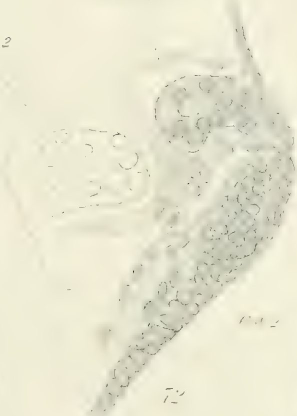
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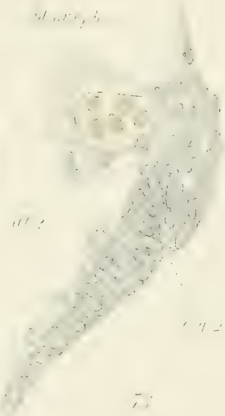
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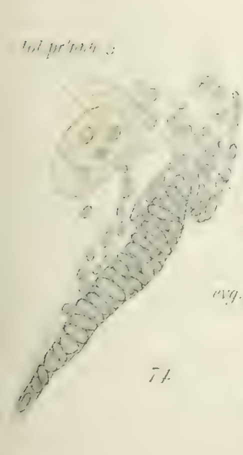

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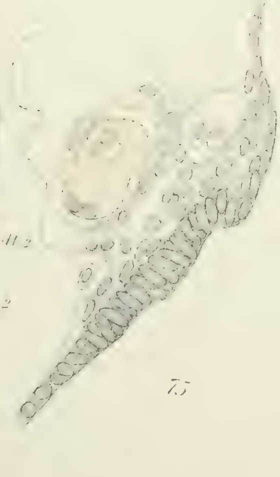
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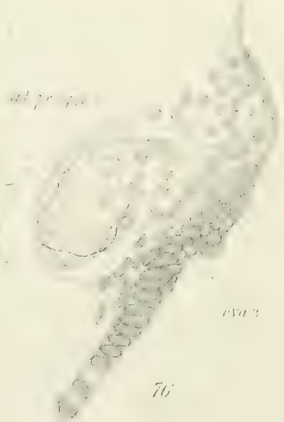
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PLATE 7.

All figures are of cross-sections.

FIG. 77. (See explanation of Plate 6.)

FIG. 78. *Hyla.* A cross-section immediately posterior to the common nephrostome and cutting the third (second + third ?) Müllerian evagination. × 308.

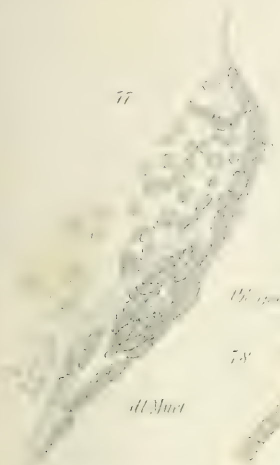
FIG. 79. *Rana.* A cross-section through the folded portion of the epithelial band of the right side of the body of a larva 35 mm. long. × 188.

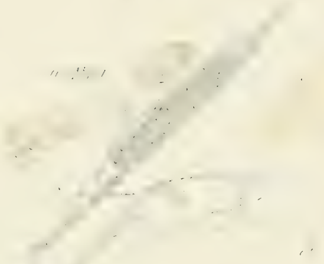
FIG. 80. *Rana.* A section behind that of Figure 79, showing the coalescence of the edges of the folded epithelial band. × 188.

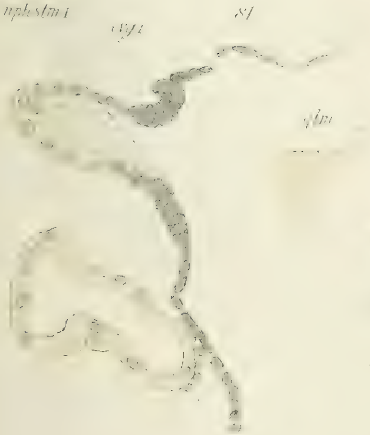
FIGS. 81–83. *Hyla.* Three sections through the right pronephros of a young larva. The first (Fig. 81) passes through the first nephrostome, cutting the fundament of the first Müllerian evagination; the second (Fig. 82) passes through the common nephrostome, cutting the second (second + third ?) evagination; the third (Fig. 83) cuts the epithelial band midway between the first and the common nephrostome. × 150.

FIG. 84. *Hyla.* A section of the second (second + third ?) evagination at an earlier stage than that of Figure 78. × 308.

FIGS. 85, 86. *Hyla.* Two sections from the left side of an abnormal larva. That of Figure 85 passes between the second and third nephrostomes (which have not fused to form a common nephrostome) and cuts the fundament of the second Müllerian evagination. That of Figure 86 passes behind the third nephrostome and cuts the third evagination. × 308.



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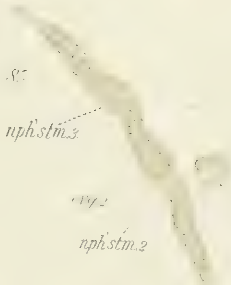
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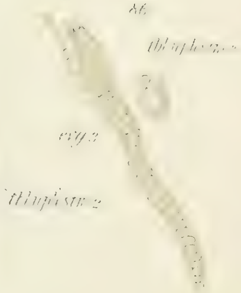
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82
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B Meisel lith Boston

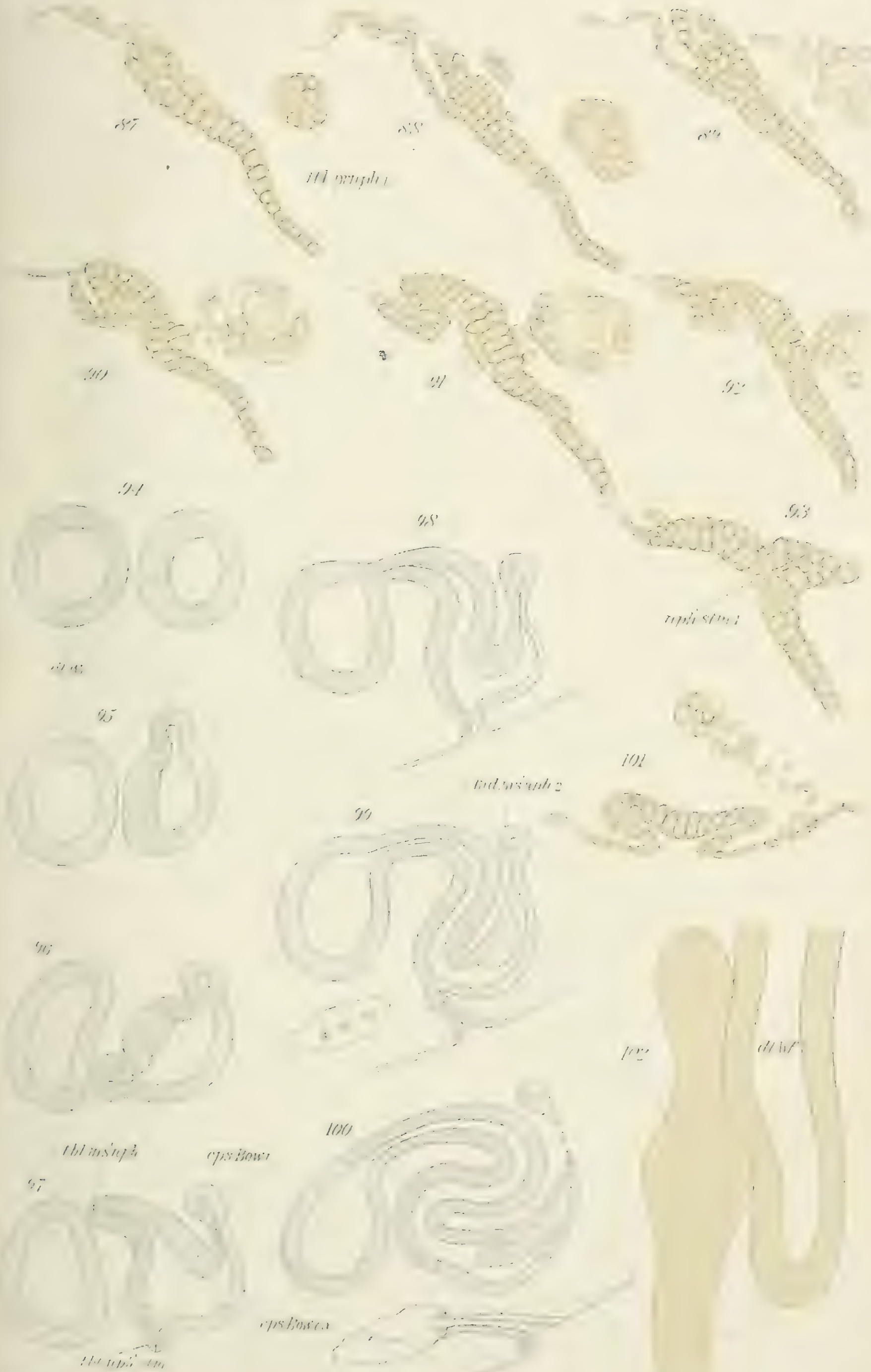
PLATE 8.

Figs. 87–93. *Hyla.* Seven cross-sections through the first evagination of the left side of the body. Figures 87–92 are of consecutive sections; Figure 93 is of the third section posterior to that of Figure 92. × 410.

Figs. 94–100. *Rana.* Seven diagrammatic figures of the anterior faces of cross-sections illustrating the development of a primary mesonephric unit of the right side of the body. The somatic portion is shaded by lines, the splanchnic by dots.

Fig. 101. *Hyla.* A cross-section through the folded epithelial band of the right side of the body. In the next section posterior to this, the edges of the fold coalesce to form the Müllerian duct. In the third posterior, the common nephrostome joins the peritoneum. It is, in this exceptional case, *dorsal* (mesal) to the opening of the Müllerian duct. × 410.

Fig. 102. *Rana.* An outline drawing, ventral aspect, of two mesonephric blastulae and the Wolffian duct from the left side of the body, made by superposing drawings of six consecutive frontal sections.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AT HARVARD COLLEGE.
VOL. XLV. No. 3.

THE OPTIC REFLEX APPARATUS OF VERTEBRATES FOR SHORT-CIRCUIT TRANSMISSION OF MOTOR REFLEXES THROUGH REISSNER'S FIBRE; ITS MORPHOLOGY, ONTOGENY, PHYLOGENY, AND FUNCTION. — PART I. THE FISH-LIKE VERTEBRATES.

BY PORTER EDWARD SARGENT.

WITH ELEVEN PLATES.

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No. 3.—CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, UNDER THE DIRECTION OF E. L. MARK, No. 153.

The Optic Reflex Apparatus of Vertebrates for Short-circuit Transmission of Motor Reflexes through Reissner's Fibre; its Morphology, Ontogeny, Phylogeny, and Function. — PART I. *The Fish-like Vertebrates.*

By PORTER EDWARD SARGENT.

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Introduction.

This research treats of a single functional unit in the central nervous system of vertebrates, its origin, history and function. The structures involved have been studied in many widely varying types, progressing from the more primitive and simpler forms to the higher and more complex. But the methods of comparative anatomy have not alone been relied upon. The evidences of development and degeneration have also been of value. An endeavor, too, has been made to attain that phylogenetic perspective which reveals necessity and environment at work in producing and modifying organic structures. It is in the beginnings of things that their secrets are laid bare. The subject of the present study is no exception, for the more primitive organisms present a simplicity of structure that makes possible an understanding of the mechanism of this apparatus that could not otherwise be attained. The optic reflex apparatus is shown to be a primitive structure, probably traceable to invertebrate ancestry, and is followed in its variations through the vertebrate series.

Aided by physiological experiment, an attempt has been made to interpret the structures described, in the belief that to the search for the more evident phenomena should be added that for mechanisms and causes. For structure and function are correlative, as are cause and effect, and our understanding of any organic structure is largely dependent upon our ability to interpret it in functional terms. The present study reveals the existence of a highly specialized nervous mechanism, by which, as the result of optical stimuli, motor reactions are by reflex activity brought about in the shortest possible time, thus accounting for the 'flight reflex' of the lower vertebrates and the quick reaction to an ocular sense of danger in the higher animals.

In the spring of 1899, while studying the central nervous system of teleosts, my attention was first attracted to a fibre or rod-like structure lying in the lumen of the canalis centralis. This was first seen in sagittal sections of the spinal cord of the trout (Salvelinus fontinalis). It could easily be traced from section to section through the canal and the brain ventricles. This rod had a very regular cylindrical form, took certain stains sharply, and had a uniform diameter of 3 micra. Further study revealed its presence in all species of vertebrates examined. Though unmentioned in the text-books and more recent literature of the nervous system, I found it referred to in the older papers as Reissner's fibre, and generally alluded to as an artifact.

The occurrence of a continuous rod or fibre in such a position was so surprising, and the sharpness and definiteness of its appearance so convincing, that I was unable to accept the views of previous observers as to its nature. I immediately devoted my time to its investigation and soon succeeded in proving that "the fibre is an organic structure, occurring in all classes of vertebrates, and intimately connected with the central nervous system which surrounds it, but within the lumen of which it lies for the most part free" (Sargent, :**00**).

Further studies on its development and cellular connections led to the discovery of a highly specialized apparatus, of which the so-called Reissner's fibre forms the conduction path, for the transmission of motor reflexes (Sargent, :**01**, :**01**^{a}).

More extended research has shown this apparatus to be one of the most archaic structures of the central nervous system, and has enabled me to trace its phylogenetic development throughout the sub-phylum Vertebrata, within which it reveals many minor changes in structure and some modifications of function.

It gives me pleasure to acknowledge my indebtedness to Prof. E. L. Mark for his able supervision and criticism of the work while in progress, and for his painstaking revision of the manuscript. I am also indebted to Prof. G. H. Parker for a number of criticisms and suggestions, and to Dr. H. C. Bumpus, formerly Scientific Director of the Wood's Hole Laboratory of the United States Fish Commission, who extended to me, while an occupant of one of the Tables controlled by the Museum of Comparative Zoölogy, opportunities for collecting a large amount of the material on which these studies are based. I desire to express my thanks likewise to Dr. H. M. Smith, the Scientific Director of the United States Fish Commission, for similar courtesies. I also acknowledge obligations to Prof. Burt Wilder, Prof. C. H. Eigenmann, and the late Dr. N. R. Harrington for valuable material which I could not have secured but for their aid.

I. Morphological.

A. GENERAL HISTORICAL SURVEY.

1. *Reissner's Fibre.*

It is remarkable that so peculiar and conspicuous a structure as Reissner's fibre, which is of so great importance in the nervous anatomy as to persist throughout the vertebrate series, should have remained for forty years after its discovery so little known. This is, no doubt, largely due to the fact that in the study of the spinal cord transverse sections, in which this rod is very inconspicuous, have been so largely relied on. However, recent investigators who have studied the central nervous system by means of longitudinal sections have also failed to note this structure, probably for the reasons that (1) many staining methods fail to bring it out clearly, and (2), being a structure of great delicacy and one of the first to break up after death, it shows clearly only in perfectly fresh material carefully fixed.

The fibre in question was discovered by Reissner (**'60**), who described it as a highly refractive cylindrical rod, 1.5 micra in diameter, lying within the lumen of the central canal of Petromyzon. "Da dieser Strang, wenn ich ihn überhaupt zu Gesicht bekam, stets von derselben Gestalt war, und nicht einmal Formverschiedenheiten darbot, welche die Axencylinder an Chromsäurepräparaten so häufig zeigen, kann ich nicht annehmen, dass er gleichbedeutend sei mit den unregelmässigen Massen, welche den Centralcanal bisweilen vollständig oder zum Theil erfüllt und im Rückenmark andere Thiere oder des Menschen von mehreren Forschern erwähnt worden sind." He denied that it could have been formed from thrown off epithelial cells or blood corpuscles, as similar contents of the canal had been accounted for by Stilling (**'59**). If, he said, it was formed from the coagulation of the albumen of the cerebro-spinal fluid, as Bidder und Kupffer (**'57**) had supposed, then one must acknowledge that the chemical composition of the cerebrospinal fluid was the same, or nearly the same, as the substance which forms the axis-cylinders. In regard to its origin and ending, Reissner could find nothing, and he knew of its occurrence in the cord of Petromyzon only.

Kutschin (**'63**) confirmed for Petromyzon Reissner's discovery, naming the structure Reissner's fibre, but failed to add anything to previous knowledge of it.

Stieda (**'68**) found Reissner's fibre present in all teleosts examined by him. He describes it as a cylindrical rod of homogeneous structure, having in general a diameter of 3.8 micra. Stieda considered it to be an artifact produced by the chromic acid used in fixation.

Later Stieda (**'73**) observed the fibre in selachians also. He says (**'73**, p. 438): "Auch hier fand ich sowohl auf Quer- als auf Längsschnitten mitunter im Lumen jenen räthselhaften Strang, welcher einem Achsencylinder im Aussehen gleicht. Ich habe den Strang fast bei allen untersuchten Wirbelthieren gesehen, und habe mich an einem andern Orte dahin ausgesprochen, dass derselbe ein Kunstproduct, d. h.

ein Gerinnsel sei. Zur unterstützung der Ansicht, dass der Strang ein Achsencylinder, d. h. ein Nervenzellenfortsatz sei, fehlt der Nachweis des thatsächlichen Zusammenhangs mit einer Nervenzellen."

Viault ('**76**) and Rohon ('**77**) saw Reissner's fibre in plagiostomes and agreed with Stieda that it was an artifact.

Sanders ('**78**, p. 742), describing the central canal of the mullet, says: "Occasionally a rod is seen in sections through this central canal of the cord, which most probably is the coagulated liquid contained therein, as Stieda suggested; it is in only a few sections that this rod is seen, for it usually falls out, not being retained in its place by any attachments."

Mayser ('**82**, p. 295) found Reissner's fibre in the fourth ventricle in teleosts and followed it forward to the region of the trigeminal nerve. He thought it might reach farther, and, contrary to the opinion of Stieda and Viault, agreed with its discoverer that it was probably preformed.

Sanders ('**86**, p. 740) failed to find the rod, or fibre, in plagiostomes, but says: "There is often found a small quantity of granular matter in the canalis centralis, which presents a granular appearance after coagulation; it corresponds to the rod occasionally found in the canalis centralis in the Teleostei, and shows perhaps that the cerebro-spinal fluid coagulates more firmly in the latter than in the former." Some years later Sanders ('**94**) found this structure of conspicuous size in Myxine.

Gadow ('**91**, p. 338) found Reissner's fibre in birds, but considered it a product of shrunken cerebro-spinal fluid and lymph corpuscles. If Reissner's fibre has been seen by other and later investigators, this view has probably been accepted by them, as I find few further references to this structure in later literature.

In July, 1899, Studnicka published a short paper on Reissner's fibre and its relation to the ventriculus terminalis. He mentions having seen the fibre in many cyclostomes, selachians, teleosts, amphibians, and amniotes, but the description is confined almost wholly to Petromyzon. He denies that the fibre is an artifact formed from the cerebro-spinal fluid, and believes it a preformed structure. He describes it as homogeneous, showing no evidence of internal structure. Some of his observations are, however, opposed to this view. In Chimaera he sometimes found a parallel splitting of the fibre, and he describes it as having in Anguilla an 'alveolar' structure. In Petromyzon the fibre, extending forward through the brain ventricles, may for a short distance penetrate the brain tissue, and again emerge into the ventricle.

This condition, he believes, is due to the fact that the brain tissue, in the course of its development, grows around and encloses the fibre. He failed to find the anterior ending, but believes that the fibre may have the power of growing forward at its anterior end. Posteriorly he finds the fibre coiled within the ventriculus terminalis. This he describes as the normal condition, at least in the adult. He apparently believes that the fibre, originally formed in the canal, may by shrinking take this convoluted form in the terminal ventricle. Later in this paper I shall describe a similar coiling of the fibre in the fourth ventricle and other parts of its course, and attempt to show it to be an unusual and abnormal condition. Studnicka denies that Reissner's fibre is a nervous structure, (1) because of its relations in the ventriculus terminalis, and (2) because it differs from an axis-cylinder in being homogeneous. Neither of these distinctions holds, as I hope conclusively to show. He concludes that Reissner's fibre is formed by the secretion of the walls of the central canal in post-embryonal development, and that the fibre does not later increase in size. I shall discuss farther on both of these points, showing them to be fallacious.

In my preliminary paper (Sargent, :00), which was prepared for the press in June, 1899, and published in January, 1900, I showed that Reissner's fibre is a preformed structure occurring in the brain ventricles and central canal of all vertebrates, and intimately connected with the nervous system. Kalberlah (:00, pp. 21–24) has criticised my view as to the nature of Reissner's fibre, on the basis of observations made by him on Acanthias embryos only. He noticed in the canal "sonderbaren Gebilde im Centralkanal" having "etwa das Bild eines Axencylinders, dessen Markscheide durch mangelhafte Fixage oder aus anderen Gründen gequollen glasig erscheint." "Da fand ich bei einem in toto im Müller gehärteten Acanthiasembryo (siehe dazu Fig. 3), bei dem auch wieder solche Pseudoaxencylinder im Centralkanal lagen, an der Peripherie des Rückenmarks, und zwar an der ventralen Seite, den Sulcus med. ausfüllend und breit anfliegend, eine ganze Kollektion solcher Fädenquerschnitte von der beschriebenen merkwürdig glasig homogenen Beschaffenheit." "Diese Fäden für physiologische Sekretströme zu halten, kann ich mich nicht entschliessen, noch weniger allerdings für präformierte nervöse Gebilde, wenigstens nach dem, was ich gesehen habe, und ich glaube sicher, dass dieselben mit dem von Sargent und den anderen beschrieben identisch sind. Ich halte die Fäden für Kunstprodukte, und zwar für herausgequollene Myelinmassen." Kalberlah's description and figure convince me that he has not seen Reissner's fibre, but has described as such artifacts formed perhaps by pressure, or rough handling, causing the myelin to flow out into the canal. In Acanthias embryos of the age studied by Kalberlah, Reissner's fibre has not yet formed in the canal (see p. 170). His studies, too, were apparently confined to transverse sections of the cord, in which it would be very difficult to identify the fibre if it were present. We may therefore safely set aside Kalberlah's conclusions, as it is improbable that he has seen this structure. Reissner's fibre has since been seen in selachians by Houser (:**01**), who confirms my findings, and by Johnston (:**02**) in Petromyzon. Kölliker (:**02**, p. 159) further confirms the results of my first paper as to the preformed nature of Reissner's fibre. Streeter (:**03**) holds to the view that it is an artifact, as: "The structure shows a marked and irregular variation in form and size in different sections; in some transverse sections it was seen as multiple 'Centralfäden'; in sections stained with toluidin blue it retains a deep blue stain, while the axis-cylinders in all other parts of the section are unstained." The appearances here described are evidently due to poor preservation of the material.

2. *The Mesencephalic Nidulus of Optic Reflex Cells.*

The nidulus of cells in the mesencephalic roof whose axons fuse to form the fibre of Reissner were first described by Rohon ('**77**) in the selachian brain, though they had previously been seen by Stieda ('**70**) in the frog, and later in the turtle. Sanders ('**86**) described the position and appearance of these cells in selachians with some minuteness. Bellonci ('**88**) noticed them in the brains of the frog and the chick, and Goronowitsch ('**88**) described cells in Acipenser which he recognized as homologous with the 'Dachkern' of Rohon. Osborn ('**88**), studying this nidulus in Amphibia, was the first to advance a theory as to its relations. Falling into error in following the neurites of the cells, he claimed that they were connected with the trigeminal nerve, and called this nidulus the 'mesencephalic trigeminal nucleus.' Burckhardt ('**91**), observing these cells in the Urodela, accepted the interpretation of Osborn, as did Rabl-Rückhard ('**94**) for the Reptilia, thus perpetuating the error. Sala ('**95**) gave an accurate but incomplete description of the cells of the torus longitudinalis of teleosts, but failed to homologize them with the corresponding cells in other vertebrates. Haller ('**98**) found in selachians and (:**00**) in Emys the 'Dachkern' nidulus, and claimed to have followed the neurites of the cells to the ventral longitudinal bundles of the medulla. Mayer ('**97**) noticed large cells in the optic lobes of Petromyzon. Johnston (:**01**) described this nidulus in Acipenser as the 'nucleus magnocellularis.' Edinger (:**01**), in a brief description of the nidulus in selachians, applied the term 'nucleus magnocellularis tecti' and reaffirmed Osborn's error by maintaining its connection with the trigeminus.

The homology of the large-celled nidulus of the mesencephalic roof with the cells of the torus longitudinalis has remained unsuspected. No one of the investigators cited, with the exception of Edinger, who saw the connection of the cerebellar neurites with the cerebellum, has traced any of the neurites correctly.

In December, 1899, I described before the Society of American Morphologists at New Haven the origin of the fibre of Reissner as the result of the fusion of the axons of the large cells of the mesencephalic nidulus, and published a brief account the following May (Sargent, :**00**^{a}). At the Baltimore meeting of the same society (December, 1900) I announced the discovery of "An Apparatus in the Nervous System of Vertebrates for the Transmission of Motor Reflexes arising from Optical Stimuli," showing the relation of the mesencephalic large-celled nidulus to the fibre of Reissner, and describing its development and physiology, a brief account of which was published the following April (Sargent, :**01**, :**01**^{a}).

Houser (:**01**) has since confirmed the anatomical details of my work as then announced, so far as they relate to selachians.

B. METHODS AND MATERIAL.

In spite of the distinctness and constancy of this fibre in the preparations where it was first seen, its unusual position made me at first incredulous as to its being a preformed structure. My first care, therefore, was to make certain that it was in no way due to the coagulation of the cerebro-spinal fluid, or the formation of other artifacts by the particular fixing agents used.

As already stated, it was first observed in the trout, which had been fixed in corrosive sublimate. Knowing the proneness of corrosive sublimate to form artifacts closely resembling organic structures, I searched for the fibre in material fixed in fluids containing no corrosive sublimate. It was found to be equally clear and sharp in material fixed in Flemming's fluid, in formol, and in many other fluids unlike corrosive sublimate in their action.

The fixing fluids were chosen so as to secure as great a diversity of composition as possible, in order to test all possible effects in the coagulation of the fluids in the central canal and the formation of artifacts in the lumen of the same. Among those used were: Flemming's stronger fluid, formol (5 to 10%), corrosive-acetic, Zenker's fluid, potassic bichromate, bichromate-formol, picric-formol-acetic, Gilson's fluid, Müller's fluid, Graf's chrome-oxalic fluid, and chromic acid. In material preserved in any of the fluids enumerated the fibre can be plainly seen, being, however, somewhat more sharply brought out by some reagents than by others.

The stains which serve best for Reissner's fibre are certain of the hematoxylins and certain of the anilines. Of the first class Mallory's phosphomolybdic and phosphotungstic hematoxylins, and Ehrlich's acetic-alum hematoxylin were the best. Iron hematoxylin brings out the fibre well, if decolorization is not carried too far. Of the anilines, methylen blue, Congo red, and acid fuchsin were found to be the most valuable. Double staining was found desirable for bringing out the internal structure of the fibre. The most successful combination was Ehrlich's hematoxylin followed by Congo red, which, by the way, is one of the best of all combination stains for the central nervous system of lower vertebrates.

Vom Rath's osmic-acid methods and the Weigert methods were also used effectively to differentiate the sheath and finer branches of Reissner's fibre. Methylen-blue impregnation has also afforded some results. Golgi methods, though tried in great variety, have so far failed to give an impregnation of the fibre. The cells, however, which give rise to the fibre of Reissner are frequently impregnated, and their axons may sometimes be followed by this method, to where they emerge into the ventricle. This failure of the fibre to take the Golgi impregnation may be explained, perhaps, by the fact that the fibre is here surrounded by the cerebro-spinal fluid, which contains a considerable percentage of non-dializable colloid substance, thus preventing the fixing fluids from reaching it readily.

In investigating the occurrence and course of Reissner's fibre, I have made and studied series of sections of the central nervous system of more than three hundred individuals representing upwards of one hundred different species, and including all the principal groups of vertebrates. In addition, I have been able to draw upon a large collection of preparations of the central nervous system of teleosts previously prepared. All the chief groups and principal sub-groups of vertebrates have been examined, and in no case where perfectly preserved material has been carefully studied have I failed to find Reissner's fibre present.

C. THE BRAIN VENTRICLES AND CENTRAL CANAL AND THEIR CONTENTS.

I. The Encephalic Cavities.

The cavities within the central nervous system, their lining and contents, have hardly attracted the attention they deserve, and this is especially true in the lower vertebrates, where they are largest. Most text-books and treatises on the nervous system make no mention of these topics, and others dismiss the subject with a few words, which have been repeated in the text-books, unmodified, for half a century. The small size of the canal and ventricles in the higher vertebrates has probably contributed to this neglect, but in the great majority of adult vertebrates and all vertebrate embryos the volume of these cavities is a considerable part of the total volume of the brain.

Magendie ('**42**) discovered the foramen which puts the intraventricular cavities in connection with the sub-arachnoid space. Key und Retzius (**'75–76**) showed that there were also two other foramina in the lateral recess by the side of the flocculus cerebelli. Haller (**:57–63**) found that colored gelatine injected into the veins appeared in the brain ventricles, and Hill (**'96**) showed by numerous experiments that, under slightly increased pressure, the cerebro-spinal fluid was absorbed into the veins, chiefly within the cranium.

All these observations have been made on the higher vertebrates only, chiefly on man and monkeys. I have been able to demonstrate the foramen of Magendie in sections of the brains of Petromyzon and teleosts. In the former it lies immediately posterior to the border of the plexus chorioidens III., and marks the anterior limit of the dorsal cells of the cord (Hinterzellen).

1. THE CEREBRO-SPINAL FLUID.

The cerebro-spinal fluid is of a clear, watery nature and low specific gravity, with only a trace of proteid. According to Halliburton (**:01**), it is a true secretion and not a transudation. "The peculiarity of cerebro-spinal fluid lies in the quality, not the quantity of proteids, that are present" (Halliburton, **'88**). These proteids constitute about one-tenth of one per cent of the cerebro-spinal fluid, and consist chiefly of globulins and albumoses (Hammarsten, **'98**, p. 195). There is about nine-tenths of one per cent of inorganic solids, and approximately ninety-nine per cent of water. The composition, however, differs in various physiological and pathological conditions. It has been shown by Cavazzani (**'92**) from experiments on dogs that the cerebro-spinal fluid collected in the morning is more alkaline and contains a greater percentage of solids than that collected at night. He considers that this is related to the activity of the nervous system, and that it confirms Obersteiner's theory of sleep. Halliburton (:01, p. 16) confirms Cavazzani's observations, and finds that in a state of fatigue there is a reduction in the amount of organic solids in the cerebro-spinal fluid.

The composition of the fluid varies, too, in diseased and normal brains. The analyses of Halliburton (:01) show that in spina bifida there is a slight increase in the amount of inorganic salts; in hydrocephalus there is a considerable increase in the amount of proteids. Mott and Halliburton ('98^a, :01) have shown that, "in the disease called General Paralysis of the Insane, the degenerative changes that occur in the central nervous system are associated with the presence of the products of such degeneration in the cerebro-spinal fluid" (:01, p. 437). Of these, choline and neurine, derivatives from the breakdown of lecithin, a constituent of protagon, have a toxic action, and when injected into the blood of another animal produce profound functional disturbances.

The cerebro-spinal fluid, Halliburton (:01) points out, has a double origin, and it is questionable if its composition is the same in all parts. He says: "It is found in the lymph channels and spaces of the brain and cord tissue, and the perivascular lymphatics have been shown to open into the sub-arachnoid space. It is found in the cerebro-spinal cavity, and it can hardly be doubted that it is here formed largely by the secretory epithelial cells which cover the choroid plexus."

In all the analyses of cerebro-spinal fluid, so far made, the fluid has been drawn from the meningeal spaces. These, being in connection with the intraventricular spaces, it has been assumed that the fluid was the same throughout. From the considerable number of characteristic secreting areas in the walls of the brain ventricles, each wholly different in structure, it seems probable that the cerebro-spinal fluid is a mixture or commingling of at least several secretions.

The researches of de Cyon ('98) and Johnston (:01) have shown not only that the saccus vasculosus is an organ for the supply of the cerebro-spinal fluid, as Rabl-Rückhard ('83) and others had previously suggested, but that its epithelial lining contains ciliate sense cells which are connected by nerve fibres with reflex centres, the whole constituting an apparatus which probably controls the supply of the cerebro-spinal fluid, and indirectly the blood-pressure and the heart-beat.

In connection with this complex question of the function of the encephalic cavities and their contained fluid, the recently expressed opinion of Minot (:**01**, p. 96) is of interest and importance: "The pineal region develops a series of structures, which, from their anatomical characteristics, appear to be directly concerned in the formation of the fluid in the cavities of the brain. We may assume that the choroid plexus supplies the main bulk of the fluid, but the gland-like organization of the epiphysis and of the paraphysis indicates that they supply by secretion special chemical substances to the encephalic fluid."

Dendy (:**02**, p. 492) has ventured the suggestion that the choroid plexus is of "importance in promoting the oxygenation of the brain-fluid," and has made some conjectures as to the means by which the fluid is circulated. The fact that the composition of the cerebro-spinal fluid varies with the state of fatigue of the nervous system also points to an important function.

2. Cilia.

The occurrence of cilia projecting into the fluid from the walls of the ventricles and canal has long been known (see Kölliker, '**96**, p. 144); but the distribution of these ciliated cells has not yet been worked out. From my incomplete observations, I believe that they have a definite but not uniform distribution. As a rule, the cilia on the roof of the canal and ventricles are larger and more numerous than elsewhere. The cilia in a larval Amia 15 mm. long are 5 micra in length, equalling one fourth the diameter of the lumen of the canal. The floor of the canal and ventricles seems to be destitute of cilia. Probably the cilia perform some function in keeping the cerebro-spinal fluid in circulation.

Johnston (:**03**^{a}) has recently observed the movements of the cerebro-spinal fluid in Cryptobranchus. "There is a general current which flows backward on the floor and the lower part of the side walls of the brain, and forward along the roof and upper part of the side walls." These currents are kept up, he believes, by the cilia.

In this connection it would be of interest to know more of the ciliated groove in the floor of the central canal, somewhat vaguely described by Beard ('**88**, p. 902), in the early development of the neural tube.

3. Ependymal Groove.

A characteristic ependymal structure lies in the roof of the diencephalon, extending from beneath the posterior commissure cephalad to the base of the ganglia habenulae. In gnathostomes this forms a median groove, generally somewhat horseshoe-shaped in transverse section. In cyclostomes there are two grooves symmetrically placed on either side of the median plane. As I have elsewhere shown (Sargent, **:03**[a]), it is a conspicuous structure in cyclostomes, selachians, and reptiles, less prominent in teleosts, birds, and amphibia, and inconspicuous in mammals. The specialized ependyma of the groove consists of characteristic elongated ependymal cells, and is sharply marked off from the usual ependyma lining other portions of the ventricle. In general it acts as a support for the constituent elements of the fibre of Reissner, which pass through it into the ventricle, and as an 'anchorage' for the fibre as a whole. This ependymal structure was, I believe, first observed by Fulliquet (**'86**, p. 100, pl. 4, fig. 17). He briefly described it as "à la partie dorsale du ventricule un boquet de cellules beaucoup plus grosses, fusiformes (*cde.*)." It has since been noticed by Rabl-Rückhard (**'87**), Edinger (**'92**), Gage (**'93**), and Dendy (**:02**).

4. Sense Organs.

Groschuff (**'97**) has described the occurrence on the floor of the central canal of groups of cells similar to the sensory buds seen in the integument of many animals. In studying the central canal in a large series of vertebrates, I have observed that sometimes the ependymal or epithelial cells forming the floor of the canal are grouped together so as to resemble such sensory buds. Such an apparent grouping of the cells is particularly likely to occur at any angle in the floor or wall of the canal. This grouping I believe to be a purely accidental occurrence of no functional significance. Occasionally in Amia an ependymal cell may have a long, pointed process resembling somewhat a sensory hair, projecting into the canal. This condition may, however, be the result of forcing the cell into the lumen by the artificial pressure applied to the outside of the cord in removing it from the animal.

II. Contained Material.

Within the lumen of the central canal and in the brain ventricles I find in the preparations that I have studied at least six distinguishable materials.

(1) *The coagulated and shrunken mass originating from the cerebrospinal fluid.* (See Sargent, **:00**, Figs. 3, 7, 9.) This fluid, according to the analyses given by Hammarsten (**'98**), contains about one per cent of solids. The coagulum may assume a great variety of forms and appearances under the action of various fixing and staining agents. It may be diffuse, finely or coarsely granular (*op. cit.*, Fig. 3), in clot-like

masses (*op. cit.*, Fig. 9), or sometimes aggregated about Reissner's fibre, whose course it may obscure, and whose diameter it may appear to increase. Sometimes, especially in the ventricles, it becomes so coagulated about a blood corpuscle or some disentegrating epithelial cell as to produce artifacts strikingly like cells in appearance. In fact, it would be possible to obtain photographs of such cell-like artifacts showing an apparent nucleus, nucleolus, and chromatin, as well as long streaming or branching dendritic processes. When such an artifact occurs about Reissner's fibre, it is easy to misinterpret it as a cell connected with the fibre. If the animal had been dead several hours before the cord and brain were placed in fixing fluid, the canal was found filled with coagulated granular matter, and the fibre was so far disintegrated as to be indistinguishable.

(2) *Detached epithelial cells.* (See Sargent, :00, Fig. 4.) These cells, I believe, are thrown out from the walls into the canal normally. I find them in all stages of being pushed out from between other cells, and in the canal they may be found in all stages of disintegration.[1] In the ventriculus terminalis some of these cells, thrown off at an early stage, persist and develop into the posterior canal cells (Plate 4, Fig. 27).

(3) *Blood corpuscles.* These are of course frequently numerous where the brain has been removed from the animal, due to artificial lesions of the blood-vessels, produced in taking out the brain; but they have also been observed in lesser number in small animals sectioned whole. Their presence may be due to minute hemorrhages produced by the fixing fluids.

(4) *Artifacts due to the fixing fluids.* These seldom occur, except in the use of corrosive sublimate, and then only when the after-treatment with iodine is not thorough. Such artifacts may have their shape more or less determined by the cavities in which they are formed, but then they are not easily misinterpreted.

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[1] Peter (:01) has described a similar process in the canal and ventricles of larval Rana. He finds that the cells of the inner pigment layer, which arises from the 'Deckschicht,' degenerate and wander into the canal or ventricle. He frequently found the broad end of the pear-shaped nucleus projecting into the ventricle, pressed out between the cells of the nerve tube, while the pointed end lay between the deeper cells. He has observed each stage of this 'Durchwanderung,' and believes this process is a dehiscence of the cells of the 'Deckschicht,' so that "das Centralnervensystem einzig und allein auf Kosten der Sinnesschicht entsteht." He thinks there is probably some relation between this process and the formation of the 'Teloderm' from the 'Leiterepithel' described by Mehnert (:96) in Emys.

(5) *The posterior canal cells,* in the ventriculus terminalis and posterior portion of the central canal. The number of these varies in different species from 6 to 40 (Plate **3**, Fig. 22; Plate **4**, Figs. 27–31).

(6) *The fibre of Reissner,* with its anterior divisions and posterior branches. (For photomicrographs of the fibre *in situ,* see Sargent, :00.) The fibre extends from the anterior end of the mesocoele caudad through the ventricles and the central canal to the ventriculus terminalis, usually lying near the floor of the ventricle or canal. Normally the course of the fibre through the ventricles and canal is perfectly straight (Sargent, :00, Figs. 2 and 3). Occasionally in sagittal sections it has an undulating course (Sargent, :00, Fig. 4), and sometimes it may be found coiled upon itself in a snarl or tangle (Plate **1**, Fig. 8), this latter condition being due to the recoil and contraction of the fibre when the cord was cut in removing the brain. Studnicka (:00) has described such a tangle formed by Reissner's fibre within the ventriculus terminalis of Petromyzon, and believes this to be the normal condition.

a. Appearance in the Fresh Condition. Not only has the fibre of Reissner been studied *in situ,* but I have been able to remove it from the canal or ventricle, and study it thus isolated with the aid of methylen-blue staining. Some of the larger selachians (Charcharias and Carcharhinus), in which the fibre has a diameter of from 15 to 25 micra, offer the most favorable subjects for such treatment. The fibre is almost perfectly transparent, but may be readily seen in a strong light because of its high refractivity. It has a considerable degree of tenacity and is somewhat elastic, so that it can be drawn across a glass slide. A number of permanent preparations were made in this way.

b. Appearance in Sections. After fixing, the fibre is usually brittle, and in cutting may break at some distance from the plane of the section. Under such conditions it breaks sharply at right angles to its length (Sargent, :00, Fig. 8). Occasionally in longitudinal sections of the cord cutting the fibre obliquely, the cut ends of the fibre are bent in the direction taken by the knife, much as a soft wire might be. Frequently in longitudinal sections the fibre is displaced in the cutting, so that it may lie close to the wall of the canal. Sometimes the deeply stained marginal portion of the wall of the canal may, in cutting, be torn off and displaced so as to give somewhat the appearance of a fibre in the canal. Such conditions may possibly account for Studnicka's conception of the nature of Reissner's fibre, he believing it to be a secretion from the wall of the canal. This, too, perhaps accounts for his statement that he has observed two or more such fibres running parallel in the canal.

In transverse sections of the spinal cord Reissner's fibre may usually be found lying near the centre of the lumen of the canal. In such sections it is inconspicuous, as it is small, and is often obscured by the presence around it of corpuscles and loose cells (Sargent, :00, Fig. 13). In thin sections the fibre is likely to drop out, since it is so slightly supported in the relatively large lumen of the canal, which is usually from ten to twenty times the diameter of the fibre; or it may become displaced and be seen in a more or less oblique position.

The fibre in transverse section is best studied in those forms where it is large, as in Cynoscion, Pomatomus, and Lophiatilus, in which it attains a diameter of 10 micra, and in some selachians, where it is even larger. In such sections the fibre shows a circular cross-section, and its cylindrical form is clearly made out by focussing. In series of transverse sections the fibre can be followed from section to section throughout its course, though it is studied to much greater advantage in longitudinal sections.

c. *Internal Structure.* The fibre of Reissner is highly organized, having a definite internal structure. It consists essentially of numerous axis-cylinders closely applied to each other, and surrounded by a single thin medullary sheath of myelin. Cross-sections of the fibre in Cynoscion, when double-stained with Ehrlich's hematoxylin and Congo red, show the central portion stained red. Surrounding this is a hyaline layer, which takes the stain very lightly, if at all. Outside this, on the periphery, is a thin medullary sheath taking a deep hematoxylin stain (Plate 7, Figs. 53, 54). This sheath is brought out still more clearly in cross-sections of the cord of the lizard prepared by vom Rath's osmic-acid method or by the Weigert methods. Here the sheath is deeply stained blue or black, the central portion of the fibre remaining unstained. The existence of this sheath is often apparent in cross-sections stained by other methods, and also in longitudinal sections. In sagittal sections of the posterior portion of the cord of the chick stained with Heidenhain's iron hematoxylin, the central portion of the fibre is seen darkly stained within the semi-transparent and lightly stained sheath. Finally, in the permanent preparations of the fibre that was withdrawn from the canal of the shark and double-stained with anilines, the sheath is found in many places wrinkled upon the fibre, and the fibrillar nature of the internal portion is clearly seen. In all its staining reactions, the sheath is similar to the medullary sheath of ordinary nerve-fibres, but is always much thinner. As there is no sheath of Schwann, this medullary sheath must be a secretion of the axis-cylinders which make up the fibre. Wlassak ('98) and Kolster ('99) have recently shown that likewise in the ordinary nerve-fibre the medullary sheath is produced by the axis-cylinder.

In addition to this medullary sheath there may be found in many species a loose membranous sheath surrounding the fibre in the anterior part of its course through the mesocoele, but never extending caudad into the fourth ventricle. It is apparently formed by the reflection upon the fibre of the membrana limitans interna which lines the ventricle. It is in no way similar to Schwann's sheath of the ordinary nerve fibre, which has recently been claimed by Gurwitsch (:00) to be formed from mesodermal cells.

The central portion of Reissner's fibre resembles the axis-cylinder in its reactions with stains, and under high powers has in section a minutely punctate appearance; under favorable conditions and by careful focussing this may be seen to be the effect of the cut ends of the axis-cylinders.

d. Size. The diameter of the fibre remains fairly constant in its course through the brain ventricles and the anterior part of the canal, but diminishes in its middle and posterior portions. Throughout the course of Reissner's fibre one may occasionally see fine fibrillae coming off from it and running toward the periphery of the canalis centralis (Plate 9, Figs. 66, 67). These have been seen both in transverse and longitudinal sections, but as they are very small, usually having a diameter of 0.1 micron or less, it is difficult to make much out of them. They are largest and most numerous in the posterior part of the canal, and at the extreme posterior end can be made out most clearly (Plate 3, Fig. 22). The diminution in the size of Reissner's fibre at its posterior end is probably due to the giving off of fine processes of this sort. In some instances these processes have been seen to pass between the epithelial cells lining the central canal into the tissue of the cord.

The diameter of Reissner's fibre varies in different groups, reaching its maximum in the teleosts and selachians. Within a given group it varies with the size and activity of the species. Among individuals of a species it varies with the size and age.

The following measurements of the diameter of the fibre and lumen of the canalis centralis show something of these relations. The measurements are in most cases given for three regions in the course of the fibre,— the 3d ventricle, the anterior part of the cord, and the posterior part.

	Third Ventricle.	Cord.			
		Anterior.		Posterior.	
		Lumen.	Fibre.	Lumen.	Fibre.
	μ	μ	μ	μ	μ
Petromyzon marinus (40 cm.)	4	—	4	—	4
Raja erinacea (10 cm.)	2.5	—	—	—	—
" " adult	6.7	—	—	—	—
Squalus acanthias	8	—	—	—	—
Carcharhinus obscurus	25	—	—	—	—
Larval Lepidosteus (1.5 cm.)	1.2	20	1.2	15	1 0.7 0.6
" Amia calva (1.7 cm.)	—	20	1	16 4	1 0.4
" " " (1.2 cm.)	—	15	1	10 4 3	0.9 0.6 0.6
" Ameiurus (2 cm.)	0.8	—	—	6	0.5
" Motella (3 cm.)	0.5	—	—	—	—
" Rhombus triacanthus (3 cm.)	0.7	—	—	—	—
Menidia notata	2.5	—	—	—	—
Naucrates ductor	2.5	—	—	—	—
Alutera schoepfii	2.5	—	—	—	1.5
Cyclopterus lumpus	—	—	3.5	—	—
Eupomotis gibbosus	—	50	3	—	—
Prionotus strigatus	—	—	3.5	—	—
Morone americana	3	—	3	—	2.5
Cynoscion regale	6	40	6	20	6
Pomatomus saltatrix	—	45	10	—	—
Lophiatilus chamelionticeps	9	90	8	—	—
Roccus lineatus	—	—	4	—	—
Stenotomus chrysops	2.5	—	2.5	—	—
Microgadus tomcod	3	—	3	—	—
Salvelinus fontinalis	—	—	3	—	—
Palinurichthys perciformis	—	—	2.5	—	—
Lophius piscatorius	—	50	5	—	—
Tylosurus marinus	—	50	2.5	—	—
Tautoga onitis	—	25	4	—	—
Opsanus tau	3	50	1.5	45	1.5
Pseudopleuronectes americana	—	30	3	—	3
Necturus maculata	3	30	2.5	—	—
Anolis sp.	—	15	2.5	12	2.5
Scelopterus undulatus (8 cm.)	—	16	1.4	12	1.2
" " (14 cm.)	—	75	1.8	15 10	1.8 1.8
Alligator mississippiensis (20 cm.)	3	30	3	25	3
Eutainia sirtalis (20 cm.)	—	15	1.5	12 10	1.4 1
Columba livia	—	25	1.8	—	—
Mus musculus	1	60	1	—	1

A comparison of the diameter of Reissner's fibre in species of widely different habits leads one to the inference that there is a direct correlation between the size of the fibre and the general activity of the animal. In a general way one may say that in the group of teleosts the fibre reaches its maximum size in the more active, swiftly moving types, of which the bluefish (Pomatomus) is a good example, where the fibre has a diameter of 10 micra. In the less active teleosts, other things being equal, the fibre is smaller. In the sluggish and inactive goosefish (Lophius), which is many times as heavy as the bluefish, the fibre has a diameter of only one micron or less.

In the light of present knowledge, after allowing for variability in size of body, habits, etc., the size of Reissner's fibre seems to hold fairly constant in the Ichthyopsida and Reptilia. In the trout and young alligator of approximately the same weight the fibre is of the same diameter, 3 micra. In the salamander (Necturus), which is somewhat smaller, the fibre is 2.5 micra in diameter. In the higher groups, birds and mammals, the fibre decreases in diameter. In the pigeon of approximately the same weight as the salamander, the diameter of the fibre is 1.8 micra. In the Mammalia the fibre seems to be still smaller, though it has not yet been studied carefully enough in this group to make generalizations safe.

D. MORPHOLOGY AND ONTOGENY OF REISSNER'S FIBRE AND ITS CELLULAR CONNECTIONS.

I. *Cyclostomes.*

A. HISTORICAL.

It was in the central canal of Petromyzon that Reissner ('60) discovered the fibre named for him, and the more important notices of it since his time have been based on the study of it in cyclostomes. He knew of its existence in the central canal only. It was not until twenty-two years later that Mayser ('82) followed Reissner's fibre in teleosts into the fourth ventricle. Sanders ('94) was the first to trace its course (in Myxine) from the ventriculus terminalis anteriad into the mesocoele, where he found it to divide into two branches. Studnicka ('99) followed it similarly in Petromyzon, but failing to discover its termination anteriorly, concluded that it was non-nervous. Johnston (:02) merely noticed its occurrence in the fourth ventricle of the brook lamprey.

B. Observations.

My studies in this group have been based on series of adult brains of Petromyzon marinus sectioned in transverse and sagittal planes. My investigations on the development of the optic reflex apparatus in cyclostomes are incomplete, and because of lack of material, have been confined to larval stages of Petromyzon planeri twenty-six and thirty days after hatching. The larvae at this stage are from 6 to 10 mm. in length.

1. Development of the Apparatus in Petromyzon. In the 26-day larvae the brain vesicles are still of large size, and the differentiation of the structures in the walls of the mesencephalon is yet incomplete. The roof in the median plane is very thin, being made up for the most part of a single layer of cells (Plate 1, Fig. 1). The region of the posterior commissure is beginning to be downfolded, and the fibres of the commissure have begun to make their way from side to side. The structures of the diencephalon are much more advanced. The ganglia habenulae with their fibre tracts are well developed and project downward into the diacoele (Plate 1, Fig. 4). The right ganglion is already of much greater size than the left. I have, however, been unable positively to identify at this stage in the right habenula the constituents which in the adult contribute to the formation of Reissner's fibre.

a. Optic Reflex Cells. Immediately posterior of the downward flexure through which the posterior commissure is to pass there is a group of cells larger than the surrounding neuroblasts, from which they have apparently become differentiated. These cells lie on either side of the median plane, and by their position and size may be recognized as the tectal reflex cells. By developing processes they are becoming multipolar. A few of them have at this early stage already begun to send out their axons obliquely into the ventricle (Fig. 1, *cl. opt. rfx.*).

In the 30-day stage (Fig. 2) the optic reflex cells have increased in number and size. They lie along the median plane in the roof of the optic lobe, close to the posterior commissure. In sagittal sections the cells in the anterior portion of the tectum are seen forming a single or double layer. In a transverse section just behind the posterior commissure these cells at this stage form a ridge projecting into the mesocoele (Fig. 3). In later stages, as the surrounding structures develop, the relations of these cells are greatly changed. The axons emerge into the ventricle in or near the median plane, so that in a strictly sagittal section a large number of axons, whose cells lie laterally, and therefore fall in other sections, may be seen entering the ventricle. Frequently, however, the axon may be seen passing directly from the cell, and occasionally the tapering end of the cell projects into the ventricle (Figs. 1, 3, *ax*). The axons are directed ventrad and caudad, and within the ventricle run together to form numerous trunks, which gradually coalesce as they continue into the fourth ventricle.

It is at this stage, about the 30th day, that the developing tissue of the cerebellum begins to grow around and enclose Reissner's fibre. In some individuals Reissner's fibre may be seen in sagittal section passing under the cerebellum into the fourth ventricle, quite free. In more advanced specimens of this age the fibre is already partially surrounded by cerebellar tissue. Studnicka also has found this free condition of Reissner's fibre in young Ammocoetes.

b. Posterior Canal-Cells. In the fourth ventricle at this stage Reissner's fibre, though very fine, is sharply outlined, and may be traced for a short distance into the central canal, where it fades away. In the middle portion of the canal I have been unable to find any trace of it. Studnicka records having failed to find it in the canal of larvae 1 cm. long. In the posterior end of the canal and in the ventriculus terminalis I find in this 30-day stage the posterior canal-cells already well developed. I have found from two to four in an individual at this stage. As in Amia, the nuclei are prominent, and possess a sharply staining nucleolus. The cytoplasm is diffuse, staining lightly. The cells are connected with the cord by dendrites. The axons run forward through the canal, and can be followed for some distance till they fade out. It is evident that at this stage of development the anteriorly running axons of the posterior canal-cells have not yet met and united with the posteriorly running axons of the tectal cells.

2. ADULT. *a. Morphology of the Mesencephalon.* The best works on the brain of Petromyzon (Ahlborn, '83, Johnston, :02) are incomplete on even the gross morphology of the brain. Moreover, there are considerable differences in the brain of Petromyzon planeri, described by Ahlborn, and Petromyzon marinus which I have studied. It is necessary, therefore, to give some preliminary account of the morphology of the middle region of the brain before the optic reflex apparatus can be intelligibly described.

Viewed from the dorsal side, the brain of Petromyzon marinus is an elongated structure having a length four to five times its greatest width. The medulla is the largest and most conspicuous structure, and in the region of the vagus lobes has a greater width than any other portion of the brain except the olfactory lobes. The sinus rhomboidalis is a wide open space covered by the greatly convoluted and vascular plexus chorioideus III (Fig. *A*). The cerebellum is but slightly developed as an independent structure. The optic lobes are large and nearly spherical. Along the median plane they are partly separated from each other by a slit-like sinus covered by the greatly convoluted plexus chorioideus II. The diencephalon is well developed and more conspicuous than in any other vertebrate brain. It consists of the two lateral elongated lobes of the optic thalamus separated dorsally by an open sinus, which is covered by the plexus chorioideus I, an almost simple membrane. The right ganglion habenula is an almond-shaped lobe, so greatly developed as to lie mostly in the median plane (Plate **1**, Fig. 4). The left ganglion habenula is much smaller and lies on the left side, partly covered by the right ganglion. The prosencephalon consists principally of two enormous olfactory lobes.

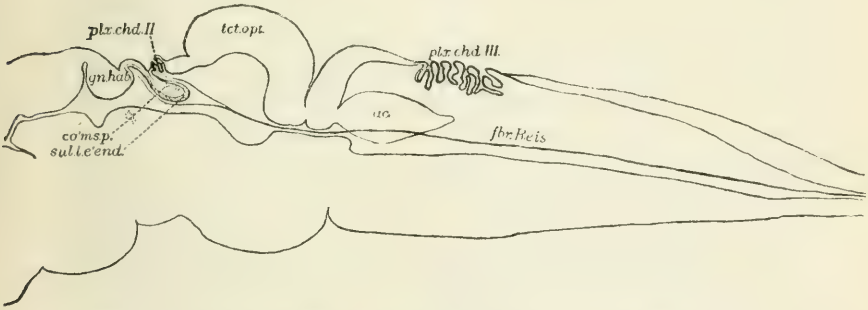

FIGURE *A*. Petromyzon marinus, adult. Nearly median parasagittal section of the brain, showing Reissner's fibre projected on the plane of section. For meaning of abbreviations, see Explanation of Plates, p. 257.

The cyclostome brain has the lateral halves of its roof less completely connected by nervous structures than that of any other vertebrate. A sagittal section of the brain shows the roof largely vascular or membranous. The only nervous structures in the median plane are the small and primitive cerebellum, the posterior portion of the mesencephalon, the posterior commissure, and the right ganglion habenula. The essentially non-nervous character of the median brain-roof and its almost complete division into bilateral halves are the conspicuous features. The plexus chorioideus II is represented in gnathostomes by the thin median roof of the mesencephalon. This relation is especially clear in such forms as Amia and Ameiurus. The sinus covered by this plexus is much wider in P. planeri than in P. marinus, so that P. marinus represents a condition in this respect intermediate between that of P. planeri and Amia.

There is no structure in the brain of Petromyzon homologous to the torus longitudinalis of the brain of teleosts. Ahlborn has represented, in the brain of Petromyzon planeri (Figs. 23, 24), a median downfolding of the mesal edge of each half of the tectum opticum, forming a pair of longitudinal ridges projecting downward into the posterior part of the mesocoele. As seen in Ahlborn's figures, the resemblance of these ridges to the torus longitudinalis in teleosts is striking. They cannot, however, in any way represent the torus longitudinalis of higher forms, for these ridges are found only posterior to the plexus chorioideus II. In P. marinus they are but little developed. They more nearly correspond in position with the corpora bigemina posterior. The tori semicirculares, projecting into the mesocoele from the lateral walls of the optic lobes, are well developed, but are not so prominent in P. marinus as Ahlborn has figured them (Figs. 24, 25) for P. planeri.

The posterior commissure crosses through the roof of the mesencephalon in a caudo-ventral downfolding of the mesencephalic roof (Fig. *A*). The mesocoele is prolonged dorsad and anteriad of the posterior commissure into a blind pocket, or recessus, which is continued cephalad in two lateral horns (Plate 1, Figs. 6, 7). The ependymal proliferation ventral to the commissure, which in gnathostomes is median, in P. marinus, following the bilateral division of the roof of the mesocoele, is in two lateral divisions. This thickened ependyma forms two longitudinal grooves (*sul. l. e'end.*, Figs. 6, 7), one on either side of the median plane, extending along the whole length of the roof of the diacoele; that of the right side is somewhat the larger, and is better developed, especially at its anterior end, corresponding in this with the greater size of the habenula of that side. Anteriorly, the ependymal grooves reach to the base of the ganglia habenulae. Posteriorly, both grooves extend along the roof of the diacoele and downward under the posterior commissure, at the same time coming nearer together. Here they curve around the commissure, which crosses the brain in a downfolding of the roof, and continue cephalad into the recessus of the mesocoele above the commissure, and thence into its anterior horns. The horns of the recessus are completely lined by this characteristic ependyma. A transverse section through the anterior part of the posterior commissure shows the horns of the recessus as small circular orifices with thick walls composed of this radiating ependyma (Plate 1, Fig. 6, *rec. l'.*).

The ependyma of these grooves is conspicuously distinguishable from the ependyma lining the other portions of the ventricles, and the demarcation between the two is always clear and sharp. It is thicker, of very compact structure, and composed of cylindrical or columnar cells with prominent nuclei (Plate 1, Fig. 6). The peripheral ends of the cells are continued peripherad in long ependymal fibres, which, collected into fasciculi, radiate from the groove. This ependymal thickening doubtless serves as a supporting structure for the axons of the tectal reflex cells which pass between its columnar cells before emerging into the ventricle. In the 26-day larvae studied no evidence of the grooves or ependyma was found. Only in the more advanced individuals of the 30-day stage could the beginnings of these structures be distinguished. It is remarkable that such definite and conspicuous structures have escaped the attention of the numerous investigators who have studied the brain of Petromyzon.[1]

Ahlborn has vaguely shown in P. planeri, in his Figure 26, a structure under the posterior commissure that may be taken to represent this ependyma, but has shown it as lying wholly in the median plane. In P. marinus this ependyma is bilateral, as already described, and nowhere united. Gage (**'93**, p. 266) first noticed this characteristic ependyma in cyclostomes, and described it as follows: "In lamprey (Fig. 110) similar cells (endyma) are found, but the masses are further separated by the post-commissure." She presents no further description, and offers no suggestion as to function further than to cite the opinion of Rabl-Rückhard (**'87**), that similar cells in Amphibia represent the torus, and the suggestion of Edinger (**'92**), that similar cells in selachians are secretory.

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[1] Since the above description was written (September, 1901), there has appeared a paper by A. Dendy, February, 1902, "On a Pair of Ciliated Grooves in the Brain of the Ammocœte apparently serving to promote the Circulation of the Fluid in the Brain-cavity." Dendy first found these lateral longitudinal grooves in the Ammocœtes stage of the New Zealand lamprey (Geotria australis), and later in the Ammocœtes stage "in one of the European species" (Petromyzon ? sp. ?). In neither of the species examined by him are the grooves so deep as I have found them in adult P. marinus. In Geotria "the inner margins of the two grooves touch one another in the middle line beneath the posterior commissure. Anteriorly the two grooves diverge from one another on the roof of the recessus subpinealis, and disappear in the deep crevices between the ganglia habenulae and the side walls of the brain. Posteriorly they terminate at the hinder margin of the posterior commissure." In neither of the species examined did he find the epithelium prolonged around and above the commissure so as to form the lining of the horns of the mesocoelic recessus, as I have described it.

Dendy has described the grooves as ciliated ; he distinguished, however, between the cilia of the ventricular walls and those of the grooves, the latter being longer. Though the ventricular walls are abundantly ciliated, I believe that there are no cilia in the grooves. He has perhaps mistaken the axons entering the grooves for cilia. He found these grooves only in the Ammocœtes stage and doubted their existence in the adult. Dendy has chosen to see some connection between these grooves and a septum formed by a fold of the choroid plexus, and believes that these perform some function in maintaining the circulation of the cerebro-spinal fluid, though he fails to make it clear in what way they could do this.

b. Optic Reflex Cells. Immediately dorsal to the posterior commissure there are two groups of large cells symmetrically placed on either side of, and at some little distance from, the median plane. These are probably the cells somewhat vaguely referred to by Mayer (**'97**). Johnston (**:02**) failed to notice these cells. In another place Johnston (**:03**, p. 1072) states that the cells corresponding to those of the 'Dachkern' are not met with in Petromyzon, although Reissner's fibre is present. They lie in immediate proximity to the ends of the horns of the recessus and partially surround them, though the greater number are ventral (Plate **1**, Figs. 6, 7). The number of these cells is not great, probably from 8 to 12 in each group. They are conspicuous in size, having a diameter two or three times that of the surrounding nerve cells. The nucleus is large, and the cytoplasm takes the iron-hematoxylin stain but lightly. These cells are undoubtedly the homologues of the tectal reflex cells which I have described in Amia and other gnathostomes. They are multipolar, and some of their neurites pass laterad with the fibres of the posterior commissure into the lateral part of the mesencephalon to the tectum, where it is probable that they are in connection with the terminals of the retinal fibres, as in other forms.

The axons of these tectal reflex cells are sharply defined non-medullated nerve-fibres, which run toward the ependymal thickening surrounding the horns of the mesocoelic recess (Fig. 6, *trt. tct. fbr. Reis.*). This they penetrate, passing between the elongated cylindrical ependymal cells, and into the lumen of the ventricle, into which they may be seen projecting radially (Figs. 6, 7). Within the lumen they unite, forming trunks, which continue caudad in the ependymal groove into the recessus and mesocoele.

There is another source of the axons which form Reissner's fibre in Petromyzon. Sharply defined unmedullated axis-cylinders come from the lateral anterior portion of the tectum opticum and run toward the median plane. Toward the median end of their course they run parallel with the fibres of the posterior commissure, from which, however, they are readily distinguished by their greater diameter and greater sharpness of outline, and by the absence of medullary sheaths. In the latter part of their course they are usually collected into small fascicles of from 3 to 12 fibres. On reaching a position dorsal to the ventricular grooves before described, they bend rather sharply at approximately a right angle, and passing between the ependymal cells enter the mesocephalic recess, where they join the trunks of Reissner's fibre (Figs. 6, *fas. Reis.*, and 7). Throughout the length of these lateral grooves in both transverse and sagittal sections, axons may occasionally be seen penetrating into the ventricle radially from between the ependymal cells, and joining the axis-cylinder-like trunks of Reissner's fibre. The cells from which these fibres arise have not always been recognized, but they are probably tectal reflex cells of inconspicuous size, which lie in the lateral portion of the tectum opticum.

c. *Habenular Constituents of Reissner's Fibre.* In Petromyzon there are still other constituents of Reissner's fibre. At the anterior extremity of the right lateral ependymal groove, at the base of the right ganglion habenula the axons of another group penetrate between the cells of the ependymal thickening and enter the groove in the roof of the diacoele (Plate 1, Fig. 5, *fbr. hab. Reis.*). Large elongated multipolar cells, lying at the base of the ganglion close to the ependymal thickening bordering the groove, furnish the greater number of these axons. Some of their neurites run toward the dorsal part of the ganglion.

A well-marked tract, consisting of a small number of sharply defined fibres, runs from the right ganglion habenula to the anterior end of the right lateral groove. This tract has its cells of origin located in the deeper parts of the ganglion. It seems to contribute some of the axons which penetrate into the diacoele.

The left lateral groove in the roof of the diacoele with its ependymal lining is not so well developed as that of the right side, corresponding in this with the dwarfed condition of the left ganglion habenula. I have been unable to distinguish any axons entering the diacoele from the left ganglion, though there may be a small number which have escaped my notice.

The axons entering the diacoele collect into a single trunk, which runs caudad into the mesocoele and at a point caudad of the posterior commissure unites with the trunk from the right horn of the recessus.

This and the trunk from the left horn continue parallel through the mesocoele to its posterior limit (Fig. *A*). Here they unite, and the resulting Reissner's fibre enters the nervous tissue of the downfolded posterior portion of the mesencephalic roof. The fibre passes through this portion of the brain and the basal part of the cerebellum in the median plane a little dorsal to the passage connecting the third and fourth ventricles. Where the fibre enters and emerges from the nervous tissue, each ventricle is continued into a minute recessus, which is prolonged canal-like for a short distance.

C. Critical Discussion.

In both the transverse and sagittal sections that I have studied Reissner's fibre has been found always to enter the *right* tuberculum acusticum (Fig. *A*). No trace of it has been found in the left acusticum. Appearances in the adult bear out the developmental evidence that in late larval life the fibre becomes surrounded and enclosed by the developing nerve tissues. In its course through the base of the cerebellum and right acusticum the fibre gives off no collaterals, and is not in direct connection with the nervous elements of these parts. It is, however, probable that this passage of the fibre through the nerve substance is, as we shall see later, of phylogenetic significance, representing a more ancestral position of this fibre tract.

Sanders (**'94**, p. 11) described in Myxine the course of Reissner's fibre, which he says is lost in the aqueduct of Sylvius. As his description is not wholly clear to me, I will quote his words: "The central canal, at first [i. e., at its posterior end] is single, but proceeding forward it shortly divides into two canals, an upper and a lower. . . . At this point both canals are surrounded by endothelium, and the rod occupies the lower of the two. From this part forward, the central canal is double, and the lower only has an endothelium of cylindrical cells, the upper being merely a space excavated in the parenchyma of the spinal cord, . . . with only a slight lining of connective tissue. At the posterior end of the medulla oblongata, the canal which is unprovided with endothelium becomes very much enlarged, and a smaller canal appears suddenly above it, which projects into the floor of the fossa rhomboidalis and contains the central rod (Fig. 7). The larger canal, on reaching the base of the fissure on the dorsal surface between the anterior end of the medulla oblongata and the posterior end of the corpora bigemina, passes downward and forward in a curved direction, and immediately beneath the posterior end of the posterior tuberosity of the brain, enlarges into a quadrangular or rounded chamber (Fig. 2), the whole looking like a pipe-bowl and its stem. The central rod comes forward from that part of the canalis centralis which is situated in the spinal cord, and passing into the enlarged canal in the medulla oblongata, there divides in two parts, one part goes straight forward in the small upper canal before mentioned on the floor of the sinus rhomboidalis, the other passes through the enlarged canal into the quadrangular chamber, and there forming a knot, goes upward, and is lost in the aqueduct of Sylvius."

Studnicka ('99, p. 7) thus describes the course of the fibre: "Bei Myxine dringt der Faden, nachdem er den engen, die Stelle des Ventriculus IV hier vertretenden Canal durchgelaufen hat, ebenfalls in die Masse des Kleinhirns und endigt in der Höhle des Mittelhirns. Der ganz enge Canal, in dem der Faden die Kleinhirnmasse durchtritt, ist nicht besonders ausgekleidet." Neither of the above investigators seems to have found the fibre embedded in the lobus acusticum, as I have described it.

The passage of Reissner's fibre for a part of its course through the nervous tissue in cyclostomes is especially interesting in throwing light on a structure in Amphioxus little understood. Of the dorsal giant nerve cells in the anterior portion of the central nervous system of Amphioxus, the largest and most anterior (designated by Rohde ('88) as the 'Kolossale Ganglienzelle, A') is median, and lies across the lumen of the central canal. It is multipolar, sending, according to Retzius ('90), several small processes to the pigment spot at the anterior end of the brain, which is generally regarded as a primitive eye. Its large axis-cylinder runs caudad in the median plane, just ventral to the canal. I believe it is probably connected posteriorly with the musculature, and is motor in function.

The direct connection of the cell with the eye, the location of the cell dorsally at the extreme anterior end of the nervous system, and the passage of the large unmedullated axis-cylinder caudad in the median plane, all suggest strongly a similarity to the optic reflex apparatus of cyclostomes. I venture the conjecture that the giant axis-cylinder of this cell in Amphioxus represents in a primitive condition Reissner's fibre of the Craniota. In Amphioxus, where the central canal is not sharply defined and the central nervous system is loosely organized, Reissner's fibre runs its whole course through the nerve tissue. In cyclostomes its course is, for the most part, through the spacious brain ventricles and canal, but for a short portion of its course it still passes through the brain tissue, perhaps a reminiscence of its past history. The fact that in Amphioxus the giant axis-cylinder in question occupies a position ventral to the neurocoele, while in the Craniota it lies within the central canal, is not the insuperable objection to homologizing the two that might at first be supposed, since in Amphioxus the neurocoele is not so definitely bounded as is the central canal in craniotes. Moreover, in that portion of the central nerve cord of Amphioxus which is immediately below the neurocoele, the nerve elements are even more loosely aggregated than elsewhere. It is easy to imagine that the giant axis-cylinder in question, in growing backward, has taken a course through this loosely organized nerve tissue rather than through the lumen of the indefinitely limited neurocoele.

On the other hand, it may be that the position of the median giant nerve fibre of Amphioxus within the nerve tissue ventral to the neurocoele presents, not a secondary and degenerate condition, but the primitive position of the original elements from which Reissner's fibre has become differentiated, and that the fibre has only secondarily, in the Craniota, shifted its position to the central canal. This hypothesis gains in importance, if we recognize the generally accepted view of Rohde and others that the giant nerve-fibres of Amphioxus and the giant nerve-fibres of the ventral nerve cord of invertebrates — of chaetopods in particular — are homologous. It is possible, then, that the optic reflex apparatus may be found in yet more primitive form in invertebrates, — that Reissner's fibre and the cells which give rise to it are represented by elements in the invertebrate nervous system.

Known facts, both anatomical and physiological, make probable such a relation between the optic reflex apparatus of vertebrates and certain of the giant fibres and cells of chaetopods.

(1) It has been shown by Spengel ('**81**), Rohde ('**87**), Friedländer ('**88**), Cerfontaine ('**92**), Vejdovsky ('**88–92**), Lewis ('**96**), and Hamaker ('**98** , that the giant nerve-fibres of chaetopods frequently are formed by the union of the neurites of two or more cells, sometimes a large number of cells.

(2) Hamaker ('**98**) and others have shown that the giant fibres of chaetopods extend for long distances through the ventral nerve cord, and by their intimate relation with other centrifugal fibres are in connection with the musculature of every segment of the body.

(3) In Nereis the giant fibres are at times parallel and in close apposition, according to Hamaker ('**98**, p. 115), "and nervous relation with other fibres is established directly between the axis-cylinders"

(p. 119). This functional relation between axis-cylinders in contact, as I shall later show, exists between the axons making up Reissner's fibre.

(4) Finally, Hamaker believes that the giant fibres, or some of them, have to do with the transmission of motor reflexes arising from optical stimuli. Hamaker (**'98**, pp. 114–115) says: "In Nereis I have frequently noted a sudden longitudinal contraction where there was apparently no stimulus except the passing of a shadow. . . . Now, if the shadow cast by a predatory animal were to bring about this movement, the mechanism would be of vital importance to the worm. Perhaps the importance of the function and the great extent of the movement brought about help to account for the large development of the giant fibres."

In the phylogeny of the optic reflex apparatus the cyclostomes form the connecting link between Amphioxus and gnathostomes in still another way. The small number of the tectal reflex cells in Petromyzon is intermediate between the one-cell state in Amphioxus and the condition in Amia, where there are nearly one hundred cells, and that in selachians, where there may be from three to four hundred. It is interesting here to note that this optic reflex apparatus has developed in direct proportion to the complexity and importance of the visual organs. Possibly some of the giant cells of Amphioxus which lie at the caudal end of the cord and send their axons cephalad are phylogenetically related to the posterior canal cells of the craniota.

I have been able to study the posterior portion of Reissner's fibre in only one adult Petromyzon. In this case the posterior 5 cm. of the tail was severed from the fresh animal; after removing the skin and muscles, the cartilaginous axis with the enclosed cord was fixed and decalcified in Flemming's fluid. The preparation was then cut into sagittal sections, and stained with iron hematoxylin. At the posterior end of the central canal, where it dilates to form the ventriculis terminalis, Reissner's fibre is found wound upon itself so as to form a tangled mass (Plate **1**, Fig. 8). This portion of the fibre has an increased diameter, and its outline is not so sharp and definite as where its course is straight through the canal. For some distance immediately anterior to the portion of the canal shown in Figure 8, the fibre was thrown into loose coils or had an undulating course, and further forward the canal was empty.

Sanders (**'94**, p. 44) has described a similar condition in Myxine: "Here it [Reissner's fibre] is particularly well developed, and has in places a curiously twisted appearance. In some specimens the chamber above mentioned, at the posterior end of the spinal cord, is occupied by a mulberry-shaped mass of glass-like aspect, from which, as from a knotted end, the rod in question emerges, in other specimens the rod is attached to the surrounding connective tissue." Studnicka (**'99**, p. 9) has also described and figured this "Endknäuel" in the ventriculus terminalis of Petromyzon and Myxine. He describes the portion of the fibre forming the knot as of indefinite outline and varicose form, and accounts for its condition in these words: "Der Knoten auf dem caudalen Ende des Reissner'schen Fadens entsteht jedenfalls nicht allein *in loco*, in dem Ventriculus terminalis, sondern der Faden schiebt sich aus dem Canalis centralis (bei den Bewegungen des Thieres?) allmählig in den Ventrikel hinein, und bildet, da er hier wenig Platz findet den Knäuel, und wird endlich in der Grundsubstanz des lockeren, von Lymphräumen stark durchgesetzten Schleimgewebes aufgelöst."

The explanation of this tangled and shrunken condition of Reissner's fibre, as I have seen it in the ventriculus terminalis, is not difficult. Observations made on the freshly isolated Reissner's fibre of sharks show that it is elastic, and tends to coil and shrink under the action of fixing fluids (pp. 144, 174). It is therefore entirely reasonable to suppose that when, in the fresh condition, the cord was severed, Reissner's fibre recoiled and shrank back into the ventricle, the posterior end being the one at which it was most firmly held. The shrinking and coiling was perhaps augmented by the action of the fixing fluid. A similar coiling of the cut end of the fibre within the fourth ventricle in teleosts is described elsewhere in this paper (p. 211).

The increased thickness and loss of definite outline may be accounted for in the same way. No such coiling and shrinking has been observed in any case where the animal has been fixed before severing the fibre. Studnicka's statement that the end of Reissner's fibre passes out of the sinus of the ventriculus terminalis and into the surrounding lymph space is so at variance with all my observations that I must believe the appearance he so interprets was accidental and due to the disturbed and abnormal condition of the fibre in his preparations.

D. SUMMARY FOR CYCLOSTOMES.

In cyclostomes the optic reflex apparatus is in many respects in a primitive condition, but the relative size of its elements shows it to be of great importance in the activities of the animal. It is relatively late in development, not being fully established until the second month of larval life. This is closely correlated with the limited activities and sluggish life of the larvae (p. 224).

The structures in the roof of the diencephalon are earlier to develop than those of the mesencephalon. In the 26-day larva the epiphysial vesicles and ganglia habenulae are already well developed. It is probable that the elements from the right habenula which contribute to the formation of Reissner's fibre have at this time sent their axons into the ventricle, though the evidence on this point is not conclusive.

The tectal reflex cells are the first elements to develop in the mesencephalon. In their early development they are aggregated in the median plane immediately behind the posterior commissure, but later, owing to the development of surrounding and intervening tissues, they are divided into two lateral groups dorsal to the commissure.

The axons of the cells passing into the ventricle coalesce, forming a number of trunks, which in their course through the mesocoele remain distinct or but loosely aggregated until a late stage of larval development. At first the fibre passes through the canal from the third to the fourth ventricle free, but during the second month the rapidly developing tissues of the meso-metencephalic fold enclose and surround the fibre so that it becomes for a portion of its course embedded in the nerve tissue. Meanwhile, during the first month, the posterior canal-cells have developed, and having established connection with the cord by dendrites, send their axons cephalad through the canal. The connection between these two intra-canal systems is not established until the second month.

In the larval Petromyzon the condition of the apparatus is very similar to that in Amia, but with the increase in morphological complexity of the adult brain this resemblance disappears. In the larvae of the first month the characteristic ependymal thickening previously described cannot be distinguished. In advanced larvae, thirty days old, the developing ependymal cells in the lateral grooves ventral to the posterior commissure are more compact and take the stain somewhat more deeply (Plate 1, Fig. 4). The ependymal grooves in the adult are conspicuously developed on both sides of the median plane, extending around the fold of the posterior commissure into the prolongations of the mesocoelic recess, and on the right side continue cephalad to the base of the right ganglion habenula. The constituent axons of Reissner's fibre pass between the cylindrical ependymal cells of the grooves before entering the ventricle. The fact that this compact ependyma is developed wherever the axons enter the ventricle and nowhere else suggests that it has some function in connection with the optic reflex apparatus. It doubtless serves as a support for the delicate axons, and possibly has further functions, perhaps nutritive.

From the base of the right ganglion habenula a group of axons emerges into the diacoele and, uniting, pass caudad into the mesocoele, where they coalesce with the other constituents of Reissner's fibre. The discovery of the habenular constituents of Reissner's fibre throws a flood of light on appearances observed in higher forms, but heretofore difficult of interpretation.

Johnston (:**01**, p. 155), after reviewing what is known of the ganglia habenulae, says, " . . . The conclusion may be drawn that in the lower vertebrates the ganglion habenula belongs to the central mechanism of the parietal eye and the olfactory organ (the relation with the parietal eye being the older ?)." It is evident that in the cyclostomes, where the eyes are poorly developed, Reissner's fibre serves as a short circuit for the transmission of reflexes arising from olfactory as well as optic stimuli. In Myxine, which is blind, having only rudimentary eyes, Reissner's fibre must be made up wholly of axons from the olfactory centre in the ganglion habenula.

II. Selachians.

In the selachians the optic reflex apparatus reaches a high state of development, perhaps, in the matter of the relative size of its elements, the highest in any vertebrate. The nidulus of cells which gives rise to Reissner's fibre is the most conspicuous element in the brain, and has been known since its discovery by Rohon as the 'Dachkern,' or roof nucleus. The cells lie in the median portion of the roof of the mesencephalon, and usually extend through the greater part of its length, though they vary considerably in their extent in different species. The first differentiation of these cells is not apparent in selachians until a much later period of development than in ganoids and teleosts. In the oviparous species the apparatus is established about the time of hatching, but in the viviparous species it appears at a relatively later stage, and is fully established only just before the animal attains a free life.

A. Historical.

Reissner's fibre was first seen in selachians by Stieda ('**73**), and somewhat later by Viault ('**76**) and Rohon ('**77**). All three agreed in regarding it as an artificial coagulation product. Sanders ('**86**) failed to find it in selachians, but noticed a granular coagulum in the canal, which he believed corresponded to it. It was not again noticed till Studnicka (**'99**) found it in Alopias, Scyllium, Acanthias (young and adult), and Chimaera; in the latter he observed a parallel splitting of the fibre.

The cells from which the components of the fibre arise were discovered in selachians by Rohon (**'77**, p. 38), and described by him as lying for the most part on either side of the median plane. He found the cells to be multipolar, each with a large eccentric nucleus and nucleolus. "Über die nähern Beziehungen derselben zu den sie umgebende Elemente vermag ich nichts Bestimmtes anzugeben. Wohl verlaufen von einer nicht unbeträchtlichen Anzahl Faserbündel, zu denen sich auch die Fortsätze von dem die Dachkerne nach aussen umsäumenden Cylinderepithel zugesellen, aber sie alle ziehen immer über, unter und zwischen den einzelnen Zellen hinweg."

Sanders (**'86**, pp. 749–751) was the next investigator to notice these cells. He described their occurrence and distribution with some minuteness in Scyllium, Rhina, Acanthias, and Raja, but otherwise added little to our knowledge of them, and fell into error in describing them as unipolar. "They generally give off one process only, or very rarely two. Rohon imagined that he saw several processes cut off close to many of these cells, and therefore came to the conclusion that they were multipolar cells, but such is not the case." Sanders traced the single process to the fibre-tracts of the middle layer of the tectum, and says of these cells, "They may be probably looked upon as corresponding to the large cells in the nerve-cell layer of the retina," and as having some function connected with the movements of the eye.

Haller (**'98**, p. 513) describes the cells of the 'Dachkern' in Scyllium as lying close to the ventricle and reaching back to the cerebellum. Though he shows the cells in a number of his figures, he adds nothing to our previous knowledge. In a later paper, Haller (**:00**, p. 277) arrives at the conclusion that the 'Dachkern' replaces ('ersetz') the 'nucleus corticalis' of teleosts, which is absent in selachians, and like it gives rise to two 'Associationsbahnen,' which run to the ventral part of the brain and are lost.

Edinger (**:01**, p. 668) noticed this roof-nucleus in Scyllium, naming it the 'nucleus magnocellularis tecti,' and described the tract of fibres arising from it and running to the cerebellum. He fell into the error of Rabl-Rückhard and Osborn, believing it to be connected with the roots of the trigeminus nerve.

Finally, Houser (**:01**), in his recent monograph, has fully confirmed my results as set forth in my preliminary papers (Sargent, :00, :01). The numerous other writers on the selachian brain have failed even to mention this great nidulus of cells.

B. OBSERVATIONS.

1. RAJA. Of this family, I have studied the optic reflex apparatus in several species, Raja erinacea, R. ocellata, and R. laevis, and at various stages of development, from the newly hatched larva to the adult. There are no essential differences between these species, but the following description applies more particularly to R. erinacea, unless otherwise stated.

a. Morphology of the Mesencephalon. The morphology of the mesencephalon is relatively simple. The optic lobes are well developed, nearly hemispherical, and separated by a shallow median dorsal fissure (Plate **2**, Fig. 12) which marks the primitive median zone. The thick walls of the optic tectum have encroached upon the median plane, so that the median zone has lost its primitive essentially ependymal structure, and, though thinner than the lateral portions of the tectum, is crowded with ganglionic cells (Plate **2**, Fig. 12; Plate **3**, Fig. 15), the most conspicuous of which are the great cells of the 'Dachkern.' The dorsal decussation of the mesencephalon carries a strong bundle of nerve fibres from the stratum medullare profundum of either side transversely across the median plane immediately above the 'Dachkern' (Figs. 12, 15, *dec. d.*).

In the newly hatched skate, 10 cm. long, the region about the posterior commissure remains in a relatively primitive state, reminiscent of the condition in cyclostomes. The posterior commissure is clearly marked off from the tectum, and in the median plane is connected with it by only a narrow bridge of tissue (Plate **3**, Figs. 15 *a*, 16). A long narrow recessus extends above the commissure and between it and the tectum opticum. Into this recessus is continued the thick ependymal surface layer of the pars intercalatus, lining it completely. This ependyma is continued from its dorsal and posterior surface as a cone-like prominence (*cras. e' end.*, Fig. 15 *β*; Plate **11**, Fig. 71).

In the adult skate the increase in the thickness of the tectum and the posterior commissure has resulted in a more intimate consolidation of these two structures (Plate **3**, Fig. 16). The recessus between them has become considerably changed and elongated. It has a long narrow neck expanding into a recess triangular in sagittal section of the brain (Fig. 16). Its anterior dorsal portion is prolonged into two conical lateral horns, similar to the corresponding structures in Petromyzon, and recalling the bilateral arrangement of the ependymal grooves in that genus. This peculiar recessus is lined throughout by the characteristic thickened ependyma of the pars intercalatus ('Schaltstück'). Posteriorly this ependyma ends in a prominence, less cone-like than in the larval condition. This ependymal thickening extends cephalad of the posterior commissure, having in cross-section the appearance of a semicircular or horseshoe-shaped arch at the summit of the narrow slit-like passage connecting the mesocoele and diacoele (Plate **2**, Fig. 14). It forms the greater thickness of the pars intercalatus, which in selachians is relatively much shorter than in ganoids and teleosts, having been encroached upon by the posterior commissure (Fig. 16). The ependymal thickening continues through the diencephalic roof into the recessus subpinealis, and in contact with the ganglia habenulae merges into the usual ependyma which lines the ventricles. The ventricular surface of this thickening is thrown into transverse folds (Fig. 16), or penetrated by finger-like recesses opening posteriorly (Plate **2**, Fig. 10). The ependyma consists of columnar cells each with a conspicuous nucleus, which is somewhat removed from the ventricular space.

b. Cells of the 'Dachkern.' The 'Dachkern' cells lie in the median portion of the mesencephalic roof, extending throughout its length immediately above the ventricle (Plate **3**, Fig. 15, *nidl. tct.*) and separated from it by only a thin layer of ependymal and neuroglia cells (Plate **3**, Fig. 18). Frequently one of the large cells is in immediate proximity to the ventricle or projects into it. Immediately dorsal to this nidulus the dorsal decussation of the tectum passes from the stratum medullare profundum of one side to that of the other (Plate **3**, Figs. 15, 18, 19, 20, *dec. d.*). The cells are most numerous through the middle half of the tectum (Fig. 15), but they extend forward in lesser number to the border of the posterior commissure (Fig. 16), and backward through the tectum quite to the cerebellum (Fig. 21). Anteriorly the cells are most numerous on either side of the median plane, where they form a two-layer nidulus connected by a lesser number of cells in the median plane. Posteriorly this bilateral arrangement disappears, and the cells form a single band in the median plane. A few straggling isolated cells may occur laterally in the tectum even at some distance from the main nidulus.

The number of cells in this roof nidulus is probably between four and five hundred. In a young specimen of R. erinacea, five hundred and seventy-two cells were counted. As portions of each cell occur in several sections, the attempt was made to count only those where the nucleolus was present, but even then the count is probably too large, as some cells have two nucleoli, and in some cases the nucleolus was probably sectioned.

In form the cells of the roof nidulus vary greatly, the form of the cell-body and the manner in which neurites are given off being determined by the compactness of the tissue in which they lie and the pressure of surrounding cells. Generally pyriform, they may be spherical, oval, or spindle-form (Plate **11**, Fig. 71). The two largest processes may come off in T-form from the tapering end of a pyriform cell, or they may come from the opposite ends of an elongated spindle cell (Fig. 18). These are the two extremes, between which there is every gradation. At the anterior end, where the tectum is thickest and where the cells are crowded together from all sides, they take a generally spheroidal, ovoid, or polygonal form, as determined by the degree of pressure from different directions. Midway in the optic lobe, where they are close to the ventricle and consequently relieved from pressure on that side, the pyriform type predominates. If closely crowded, they may have a cuboidal or polygonal form. At the posterior end of the tectum, where the few scattered cells lie amid the strands of the fibre-tract passing to the cerebellum, the cell necessarily takes an elongated spindle form, the degree of elongation being determined by the closeness of the fasciculi of fibres between which they lie. Similar variations in the form of these tectal cells occur in birds, where the cells have a similar distribution. On the other hand, in Amia, where the tectal reflex cells are concentrated at the anterior end of the tectum, and the conditions of pressure, etc., are more uniform for all the cells of the group, these cells are almost uniformly spherical, tending to the polygonal outline. These variations merely indicate that the external form of the nerve-cell has little significance, except that it is the expression of all the external forces to which the cell is subjected.

Numerous capillaries in the roof of the optic ventricle, ramifying among these cells (Figs. 15, 18), supply them with an abundance of blood, and bear evidence of their activity. At the anterior end of this series, near the posterior commissure, I have observed a considerable number of these tectal reflex cells which are apparently undergoing atrophy and degeneration, showing all the stages in the process that have been observed in the atrophy of the dorsal giant cells of the spinal cord. This degeneration probably affects only a few of the many cells of this group. As yet I am totally ignorant of its meaning.

Occasionally, especially in the middle cells of the series, I have seen a direct anastomosis of adjoining tectal reflex cells, a dendrite passing directly over from one into the body of another cell. The difficulty of establishing such anastomoses even where they abundantly exist, leads me to believe that between these cells they may occur frequently.

The tectal reflex cells lie among the coarse ependymal fibres (Figs. 18, 20, *fbr. e'end.*) which arise from cell-bodies in direct contact with the ventricle and radiate from the median roof of the mesencephalon. Each cell is surrounded by a loose pericellular capsule of finer neuroglia fibres (Fig. 19, *cps. pi'cl.*). Other fine fibres, probably nervous, penetrate this capsule and branching minutely end in anastomosing arborizations in contact with the cell-body (Figure 19, *a*). According to Houser, these fibres are from the stratum medullare profundum. This seems entirely probable, though I have been unable to trace them to their origin.

Turner and Hunter (**'99**, p. 123) have described such a network covering the cell-body of a nerve-cell, and they regard it as the terminal apparatus of the axis-cylinder of another nerve-cell, and Meyer (**'97**, p. 475) has described the basket-like ending of an axis-cylinder over the body of a nerve-cell. Held (**'97**) has shown that in the case of many of the cells of the pons, cerebellum, and cord of the rabbit, it is possible to trace a growing intimacy of union between terminals of axons and cell-bodies. About the time of the birth of the animal there is simple proximity of a group of fibrils, the terminals come into contact with and gradually fuse with the cell-body by a process that Held calls 'concrescence.' For some time the junction remains marked by a layer of more highly refractive substance, but in some cases this too disappears, and the fibrils, which may penetrate far into the cell-body, can be distinguished from it only by their slightly different texture and staining reactions. My preparations lead me to believe that I have here found an intermediate stage in the concrescence described by Held, as some of the fibrils of the arborizations penetrate within the surface of the cell-body.

In size the tectal reflex cells of Raja greatly exceed any other cells in the brain. In young specimens of R. erinacea 12 centimetres long they have generally a diameter of from 25 to 40 micra. The elongated spindle cells are much greater in length. The nucleus is large and clear, 10 to 12 micra in diameter, or nearly one-third the diameter of the cell. It contains one or more spherical nucleoli, 2 to 3 micra in diameter, which are usually eccentric in position, lying nearest that side of the nucleus which is nearest to the boundary of the cell.

In the adult the cells are much larger, the maximum diameter being frequently as much as 100 micra. The nucleus is always eccentric in its position, sometimes so much so that it lies in a slight protuberance from the body of the cell, as was noted by Houser (:**01**, p. 130). As in Amia, it is always located at the side of the cell opposite to that from which the axon arises (Plate **3**, Figs. 18–20). For instance, in the middle cells of the series, where the tapering process passes off dorsally, the nucleus always lies at the ventral side of the cell close to the third ventricle (Figs. 18–21). Occasionally a cell in this region sends its axon directly cephalad, in which case the nucleus is found at the posterior end of the cell (Fig. 21). Again, near the posterior commissure, where the axon may pass directly ventrad, the nucleus is eccentric and dorsal. This, it seems to me, precludes the possibility that gravity plays any part in determining the position of the nucleus, and forcibly suggests a definite law in the relation of these parts, at least for these cells.

The cytoplasm appears minutely granular under the highest powers, due, as suggested by Houser, to the numerous small Nissl's granules. The centre of the cell is, however, freer from these than the periphery, where the granules are larger and more numerous (Fig. 19).

As already implied, the cells are usually multipolar, giving off, in addition to some finer dendrites, three principal processes (Plate **3**, Figs. 19, 20). Frequently, however, the cells are bipolar, or even unipolar in appearance (Figs. 18, 21). In the pyriform unipolar cells the single process divides in T-form to give rise to the axon and cerebellar neurite. The shortest of these three processes passes more or less directly laterad and dorsad with the fibres of the dorsal decussation (Figs. 18, *ax′*, and 20) toward the ectal region of the tectum opticum, and is lost in the stratum medullare profundum, where it comes directly in contact with the endings of the proximally running fibres of the optic nerve. It is by this process that the cell is put in direct connection with the outer world through the retina.

A second and much larger process forms, with others, the tractus tecto-cerebellaris (Fig. 18, *ax″*). Usually this comes off from the cell separately, but it may arise by the division of the single process. In the polygonal or elongated cells in the posterior portion of the tectum it passes off directly from the caudal end of the cell (Fig. 18). In the anterior cells it more frequently arises by division. Whatever its manner of origin, it takes a course laterad and dorsad from the cell, and turning caudad joins with other similar fibres to form one of two fibre-tracts on either side of the median plane (Plate **2**, Fig. 12, *trt. tct. cbl.*) which run caudad into the cerebellum (Plate **3**, Fig. 15). These neurites are coarse, sharply defined, and unmedullated (Fig. 18). They are collected into fasciculi, in which the separate fibres may be distinctly followed (Fig. 15). These fibre-tracts run caudad through the tectum, on either side of the median plane, and at some distance from it (Plate **2**, Fig. 12). Near the posterior limit of the tectum these two lateral fibre-tracts, still composed of many fasciculi in which the separate fibres are clearly distinguished, converge, bend downward, and passing under the trochlearis commissure, and through the narrow bridge of connecting tissue enter the cerebellum (Plate **3**, Fig. 15). A part at least of these fibres decussate in the base of the cerebellum (Fig. 15, *dec.*), passing to the opposite side of the brain from that in which they arise. The fibres, here separating, ascend and distribute themselves through the basal fibre-layer of the cerebellum (Fig. 15). In this anterior median portion of the cerebellum the granular layer is absent or thin, and the Purkinje cells are in direct contact with this fibre-layer. The fibres of the tractus tecto-cerebellaris probably end in fibrillations in the molecular layer of the cerebellum.

The third set of neurites from these cells, which for distinction I have called the axons (Figs. 15, 18, ax'''), pass by more or less direct paths to the anterior end of the mesencephalon, where they enter the ventricle in a manner yet to be described. The axon is finer and more delicate than the cerebellar neurite, and more difficult to follow. These axons may arise from the cell independently, or by division of the single nerve-process. From the posterior cells the axons pass cephalad, turning slightly laterad and dorsad (Fig. 18, ax''') to form two loosely aggregated fibre-tracts, which run forward through the tectum on either side the median plane (Fig. 12, *trt. tct. fbr. Reis.*). From the middle cells of the series the axons usually arise by a splitting of the T-shaped process, and pass laterad and dorsad, joining the lateral fibre-tracts which run anteriorly. This tractus tecto-fibrae Reissneris runs immediately below the dorsal decussation (Figs. 15, 16) somewhat dorsal and mesal of the tractus tecto-cerebellaris (Fig. 12). In the anterior portion of the tectum these two fibre-tracts converge toward the median plane, the separate axons meanwhile uniting into fascicles and becoming more closely consolidated. The bundle of fascicles thus formed continues ventrad as far as the level of the posterior commissure, where it curves posteriorly and enters the ventricle to form Reissner's fibre (Fig. 16).

The exact method by which the fascicles enter the ventricle and form Reissner's fibre has been difficult to make out completely in Raja, the connections having been broken away in all my series of sections. In one series of Raja erinacea, shortly after hatching, the fascicles, united for the most part in one trunk, emerge into the recess above the commissure. At the point of emergence the ependymal thickening is continued outward over the fibre some little distance in a cone-shaped prominence (Plate **11**, Fig. 71). Some fine branches emerge into the recess through the ependyma at other points, uniting with the fibre of Reissner in the ventricle. In another series of the adult brain some fine branches of Reissner's fibre are to be found extending through the narrow recess into the ventricle (Fig. 16). Some fine branches are also found beneath the pars intercalatus and apparently come from the habenular region.

All these branches unite in the anterior portion of the mesocoele, forming Reissner's fibre, which runs caudad through the ventricles and into the central canal. It lies near the floor of the ventricles and follows a straight course through them. In young individuals of R. erinacea its diameter is from 2 to 3 micra; in the adult it may have a diameter as great as 6 or 7 micra.

2. SQUALUS ACANTHIAS AND MUSTELUS CANIS. *a. Development.* A series of Squalus acanthias, from embryos 10 mm. long to the adult, has been studied. The optic reflex apparatus is late in development. In embryos from 10 to 30 mm. in length, no trace of the fibre of Reissner is to be found, and the cells of the optic tectum are as yet undifferentiated. The first evidence of a differentiation of the 'Dachkern' cells from the neuroblasts of the optic tectum was observed in an embryo 35 mm. long. Certain of the neuroblasts lying close to the ventricle in the median portion of the tectum, and destined to form the 'Dachkern' cells, are then distinguishable from the surrounding cells. In later stages (40–50 mm. long), in which the layers of the tectum are differentiated, these cells increase in size and conspicuousness, staining more deeply than the surrounding cells, but no distinguishable processes have yet formed (Fig. 17, *nidl. tct.*). In the 'pup' just before birth the cells and processes are well developed, and have attained nearly the adult condition.

In embryos from 30 to 50 mm. in length, in which the 'Dachkern' has not yet developed, there may be found a delicate fibre running through the ventricles and into the central canal (Plate **3**, Fig. 17). This has been traced forward under the 'Schaltstück,' and it probably arises from the ganglia habenulae. It doubtless consists of those axons which arise from the habenulae and join the fibre of Reissner. As the embryos of Squalus are retained within the body of the mother until they reach a length of 15 to 20 centimetres, the visual centres are retarded in development, but the habenular axons, developing at the normal time, are for a period the only constituents of Reissner's fibre, and not until just before birth are they joined by the axons from the optic centres.

As the embryos have a considerable power of movement within the mother at even an early stage, we may readily imagine that this incomplete fundament of Reissner's fibre functions at an early stage as a direct path for reflexes between the habenular (olfactory ?) centres and the musculature.

b. Adult. (1) *Morphology of the Mid-brain Roof.* In Squalus acanthias the optic lobes when viewed from above are semicircular in outline; in Mustelus canis they are larger and elliptical, the increase in size having resulted in a bulging forward of the lobes. A sagittal section through the brain of Mustelus (Plate 2, Fig. 9) shows the arched roof of the mesencephalon carried forward and projecting above the diencephalon. In the median plane the posterior commissure is not separated from the tectum as in Raja, but passes through its anterior portion (Figs. 9 and 10). The more intimate consolidation of the posterior commissure and the tectum opticum has resulted in a great modification of the recess, — which in Raja extends above the commissure, — amounting almost to its obliteration (compare Fig. 10 with Plate 3, Fig. 16, *rec. ms'coel.*). A careful study of the figures suggests strongly that the condition in Raja is a degenerate one, — perhaps a return to the primitive condition of Petromyzon, — due to the shrinking of the tectum, as the visual apparatus has decreased with the changed habits of this group of fishes. In Mustelus the ependymal thickening (*e'end.*) has attained a most conspicuous development (Plate 2, Figs. 9, 10, 14). Perhaps its increase in thickness and lateral extent is due to its longitudinal restriction by the obliteration of the recess above the posterior commissure. Under the commissure it has in cross-section a horseshoe form standing out prominently from the ventricular wall (Fig. 14). It forms an almost complete tube, a longitudinal slit in its ventral wall extending from the anterior limit of the posterior commissure to the ganglia habenulae and the base of the epiphysial stalk. A parasagittal section consequently cuts the ependymal wall of the tube in two places. In Figure 10 a long stretch of the ventral lip of this tube is shown with an adherent portion of the ventricular wall (β) tangentially cut. Figure 9 is a median sagittal section, except under the middle portion of posterior commissure, where the plane of section being somewhat lateral cuts the ventral lip of the ependyma (β). This median section shows behind the commissure the greatly reduced recessus (Fig. 9, *rec. ms'coel.*)

extending dorsad, but not far enough to lie on its dorsal side as in Raja (Fig. 16). The ventral portion of this figure is strictly median.

At its posterior end (Fig. 10, *e'end.*) the demarcation between this specialized ependyma and that which lines the rest of the mesocoele is abrupt. At the anterior end there is a more gradual transition into the vascular epithelium of the epiphysial stalk.

(2) *The Cells of the 'Dachkern.'* The cells of the 'Dachkern' lie in the mesal portion of the tectum on either side of, and in, the median plane (Fig. 9), but are incompletely separable into two lateral niduli. The cells lie close to the ventricle, immediately above the ependymal lining of the roof. Near the median plane they form but a single layer (Fig. 9), but more laterally two or three cells may be superposed (Plate 3, Fig. 21). They extend from the extreme posterior part of the tectum, in contact with the cerebellum, cephalad to within a short distance of the posterior commissure, but are more numerous and attain greater size in the posterior two-thirds of the tectum. The cells seem to have a tendency to migrate posteriorly. A few are always to be found beneath the trochlearis commissure (Figs. 9, 11, *a*), as has been noted by Haller ('98). Their number is considerably less than in Raja, probably not exceeding two hundred. Since they are less crowded, they are more regular in form (Fig. 21). The spherical or ovoid form, modified by the multipolarity of the cell, predominates. The optic lobes of Mustelus are larger than those of Raja, and the 'Dachkern' cells are of proportionally greater size, attaining a maximum diameter of 200 micra.

The nucleus is of sharper outline than in Raja, and almost uniformly spherical. It is eccentric and occasionally, as in Raja, is pushed far out from the centre of the cell so as to form a protrusion. It contains one or more nucleoli, around which the chromatin forms a reticulum (Fig. 21). The cytoplasm, as in Raja, is minutely granular, but near the surface of the cell the Nissl's granules are more numerous and of larger size. The neuroglia capsule surrounding the cell is not so markedly developed as in Raja.

Upon cursory examination the cells appear unipolar or bipolar (Fig. 21, *a*, *c*). More careful study shows them to be multipolar, as in Raja, though the number of fine dendrites arising from the body of the cell is less than in Raja. Frequently the axon and cerebellar neurite arise from the T-shaped division of a single process, but more commonly they come off from the opposite ends of the cell (Plate 2, Fig. 9). The typical cell is ovoid, its long axis lying parallel with the roof of the ventricle. The two neurites arise at the level of the dorsal surface of the cell from its opposite ends (Plate **3**, Fig. 21, *c*, Plate **2**, Fig. 9).

The tractus tecto-cerebellaris (Fig. 9, *trt. tct. cbl.*), formed from the posteriorly directed neurites of the 'Dachkern' cells, consists of several small fascicles of large fibres, and runs caudad through the tectum on either side of the median plane and some distance from it. At the posterior end of the tectum these lateral tracts (Fig. 11, *trt. tct. cbl.*) converge toward the median plane and, passing under the commissure (*coms. IV*) formed by the decussation of the roots of the trochlear nerve, pass upward into the basal fibre-layer of the cerebellum. The fibres of the tractus tecto-cerebellaris, for the most part, decussate (Fig. 11, *dec.*) before entering the cerebellum, but some of them apparently pass directly into the cerebellum without decussation. A few cells of the 'Dachkern' may always be found in Mustelus, ventral and posterior to the trochlear commissure, apparently crowded backward so as to lie almost within the base of the cerebellum (Figs. 9, 11, *α*).

The axons of the 'Dachkern' cells which run cephalad arise either from the anterior end of the cell, or by the division of a single dorsal process (Fig. 9). They are finer than the cerebellar neurites and less easily followed. Passing dorsad and laterad from the cells, they take a course cephalad, forming the tractus tecto-fibrae Reissneris (*trt. tct. fbr. Reis.*) on either side of the median plane, immediately below the dorsal decussation of the tectum (Fig. 9, *dec. d.*) In the anterior portion of the tectum these tracts converge toward the median plane and pass under the posterior commissure toward the ependymal groove (Figs. 9, 13).

Among the finer fibres of these tracts may be seen coarser ones (Fig. 13), apparently formed by the fusion of several or many axons. Numerous ependymal fibres here make it difficult to follow the individual nerve-fibres of this tract. Near the upper limit of the ependymal groove the fibres are lost, but apparently they pass between the ependymal cells into the groove. In the process of section-cutting the branches of Reissner's fibre are usually broken from their connections with the brain tissue. However, in one series I have found Reissner's fibre in place within the groove (Fig. 10). The constituent divisions of Reissner's fibre enter the groove through the thickened ependyma; and where the larger divisions enter, the ependyma is indented, forming a conical pocket (Fig. 10, *a*, *a*). A direct connection between the fibre-tracts described and Reissner's fibre has not been observed in this species. However, we may conclude that the axons of the tractus tecto-fibrae Reissneris, consolidated into a few main trunks, enter the ventricle ventral to the posterior commissure, passing through the ependymal thickening, which probably serves as a supporting structure and 'anchorage' for the fibre. In the anterior portion of the ependymal groove (Fig. 10, *sul. e'end.*) one or two fine trunks have been traced from the base of the ganglia habenulae (*gn. hab.*) caudad in the groove to where they unite with the anterior divisions of Reissner's fibre.

Just caudad of the posterior commissure all these constituent trunks unite to form Reissner's fibre (Fig. 10, *fbr. Reis.*), which from this point is easily traced caudad through the ventricles and central canal. Its diameter in the adult Squalus is 6 to 8 micra, but in the dusky shark, Carcharhinus obscurus, 8 ft. long, it attains a diameter of 25 micra. In one series of sections from the posterior portion of a Squalus embryo, 5 cm. long, I have found Reissner's fibre coiled within the posterior end of the central canal, in much the same condition as that described for Petromyzon (Plate 1, Fig. 8).

In its course through the canal, particularly in the caudal portion, Reissner's fibre gives off fine branches. These are generally directed obliquely caudad and in traversing the canal frequently undergo division; the several resulting fibrils enter the cord between the ependymal cells of the latero-ventral walls of the canal (Plate 9, Fig. 67).

c. Posterior Canal-Cells. At its posterior extremity the central canal abruptly dilates into a large oval, or nearly spherical, terminal ventricle (Plate 3, Fig. 22). In embryos up to 5 cm. in length there remains an out-pocketing at the caudo-ventral extremity of the ventricle, the remnant of the neurenteric canal (Fig. 22, *can. n'enr.*). Within the ventricle, for the most part in its ventral half, there are from six to eight posterior canal-cells, and within the adjacent portion of the canal there are from ten to twenty additional cells of much smaller size (Plate 9, Fig. 67; Plate 3, Fig. 22). The cytoplasm is diffuse, taking stains lightly. The large oval nucleus is sharply defined, and usually has a single nucleolus, around which the chromatin is arranged in a reticulum. The cells are multipolar, their several dendrites passing into the wall of the canal or of the terminal ventricle. The axons, in passing cephalad through the terminal ventricle and canal, unite with one another. In the posterior portion of the canal they eventually fuse with Reissner's fibre. Figure 22 shows the character and relations of these cells. It is a composite drawing made from four successive sections. Each section was drawn by camera in outline on tracing-paper, and the outlines superposed to get the relative positions of the cells.

C. Critical Discussion.

The ependymal groove in the diencephalic roof, with its characteristic thickened ependyma, seems to have generally escaped the observation of the numerous investigators of the selachian brain. The only notice of it in selachians that I have been able to find is by Edinger (**'92**, p. 20), who regarded it as a modification of the 'Ventrikelepithel,' having some secreting function. "Im dorsalen Gebiete innerhalb und besonders caudal von der Habenularegion treten dicke Zellen auf, die einen kürzeren oder doch weniger deutlichen Fortsatz besitzen. Von diesen Zellen wird eine Art Rinne gebildet, die den Ventrikel von oben her abgrenzt. Hinter der Commissur schliesst sich diese Rinne zum Epiphysenschlauche. Gleich darauf tritt die, bei dem untersuchten Examplare auffällig dünne, Commissura posterior auf. In der erwähnten Rinne finde ich eine geronnene gleichförmige Masse, die ganz so aussieht als würde hier eine Art Sekret in die Hirnhöhlen ergossen."

The thickened ependyma doubtless has for its principal function the support of the fine end-branches of Reissner's fibre, the 'anchorage' of the fibre as a whole serving much as the inlet board at a central station does for the telephone wires entering the building. The ependyma itself is firmly held in place by strong bundles of radiating ependymal fibres, which run from the ependymal cells to the dorsal surface of the brain.

Rohon's original description of the 'Dachkern' in selachians was accurate so far as it went, and is completely substantiated by my results. He saw clearly that the cells were multipolar. "Die Zellenfortsätze gehen grösstentheils zufolge des bedeutenden Zellumfanges während der Schnittführung verloren, und man wäre in vielen Fällen geneigt anzunehmen, dass hier bipolare, den Spinalganglienelementen ähnliche Zellen zum Vorschein kommen. Dem ist aber nicht so, weil man bei eingehender Beobachtung fast an jeder Zelle mehrfache Stellen der abgeschnitten Fortsätze sieht, und ausserdem in einigen Fällen starke Fortsätze nach gewissen Strecken hin von der Zellsubstanz verfolgen kann. Diese grossen Nervenkörper der Dachkerne müssen demnach als multipolare Ganglienzellen angesehen werden" (p. 38). Sanders (**'86**, p. 750) emphatically denies this. He says, "They generally give off one process only, or very rarely two. Rohon imagined he saw several processes." Subsequent observers — Haller, Edinger, and Houser — have described but a single process. Both Rohon and Sanders traced a process from the cells into the middle fibre-layer of the tectum.

In his paper on the brain of Salmo and Scyllium, Haller ('**98**, p. 514) was unable to arrive at any conclusions as to the relations or meaning of the 'Dachkern.' In his paper on the brain of Emys (:**00**, p. 277) he claims that the 'Dachkern' is the analogue of the 'nucleus corticalis,' and that the axons of the cells join the 'Associationsbahn' which runs ventrad to the Oculomotor region, where it decussates with the corresponding bundle of the opposite side and is lost between the ventral longitudinal bundles.

In describing the termination of the optic tract, Houser (:**01**, p. 125) shows that "the fibres of the stratum medullare profundum pass into the base of the midbrain in two somewhat clearly marked divisions. The inner division is composed of those fibres lying nearer to the central gray matter from the outset. Some of these have but a short course downward and outward, but the great mass of fibres continues near the median line as a series of intercrossing bundles which are destined to decussate ventral to the aqueduct of Sylvius."

A comparison of Houser's description and figures with those of Haller shows that Haller's 'Associationbahn' is the "inner division of the stratum medullare profundum" of Houser. It is easy to conceive, then, how Haller, having traced the axons into the neighborhood of the stratum medullare profundum, should have been misled as to their destination.

Edinger (:**01**, p. 668), in describing in Scyllium the fibre-tract from the tectum opticum to the cerebellum, alludes to 'Dachkern' as follows: "Seine dicken Markfasern sammeln sich aus der tiefsten Schicht markhaltiger Fasern, welche das Mittelhirndach besitzt, dicht über den grossen Zellen des Nucleus magnocellularis tecti, den man gewöhnlich dem Trigeminus zutheilt indem man ihn dem Mittelhirnkern dieses Nerven, der für die Säuger ausser Frage gestellt ist, homologisirt. Seine Lage spricht für diese Auffassung, dennoch halte ich es für durchaus möglich, dass diesen Zellen die Fasern des Tractus cerebello-tectalis entstammen."

Houser, having failed to discover the two principal processes arising from the cells of the 'Dachkern,' naturally failed to distinguish the axon and cerebellar neurite. He says (:**01**, p. 130): "The axons from the several cells of the same side turn . . . anteriorly, and become associated into bundles, which constitute a fairly well marked tract. This tract extends forward to the anterior limit of the midbrain, where it unites with its fellow from the opposite side, and the united group of fibres emerges from the midbrain roof to penetrate the aqueduct of Sylvius as the fibre of Reissner. Those cells of the roof-nucleus lying in the posterior region have a different termination for their axons from the one just described. In this instance the axons pass posteriorly, instead of anteriorly, and they take a course into the cerebellum." As I have before stated, I believe that each cell sends an axon anteriorly and also a cerebellar neurite posteriorly into the cerebellum.

The tractus tecto-cerebellaris has been observed in selachians by Sanders ('86, p. 749) and Haller ('98, p. 514), but they both failed to recognize its connection with the 'Dachkern.' Its origin was discovered independently and perhaps simultaneously by Edinger (:01) and Sargent (:01^a). Of its termination in the cerebellum, Edinger says merely: "Der ganze Zug, der ürbrigens aus nur relativ sehr dicken Fasern besteht, verschwindet im Velum anticum caudad von der Trochleariswurzelkreuzung."

In one other particular Houser's observations differ from mine, — as to the nature of the connection between the cells, and the optic fibres. Houser has described "nerve-fibres emerging from the stratum medullare profundum and terminating in arborizations near the bodies of the nerve-cells." He fails to mention any other mode of communication. Although I have seen and described (p. 167) such fibres, terminating in arborizations over the bodies of the cells, and think it quite probable that they come from the stratum medullare profundum, I believe the more essential connection between the two is by the dendrites arising from the cells of the 'Dachkern' and terminating in the stratum medullare profundum.

Houser (p. 139) evidently believes that the olfactory centres are connected with Reissner's fibre. "The neurones of the nucleus strati grisei are, primarily, a relay in the olfacto-motor chain. . . . The axones from the cells of the nucleus strati grisei pass backwards into the base of the midbrain as the tractus thalamo-tectalis, and then sweep upward into the tectum to lie in the stratum medullare profundum. Here they are associated with other sensory nerve-fibres, . . . and the entire group becomes related to the remarkable motor-conducting path provided by the cells of the roof-nucleus and the fibre of Reissner." But his evidence for this is not given in detail. I have recorded observations elsewhere in this paper which lead to the belief that there is a connection of the olfactory centres with the fibre of Reissner, but in a very different way from that suggested by Houser.

D. Summary for Selachians.

Judged by the relative size of its elements, the optic reflex apparatus reaches perhaps its highest development in the selachians. Its cell nidulus, known since the time of Rohon as the 'Dachkern,' extends on either side of the median plane through the length of the optic lobes, close to the ventricle.

The cells of the 'Dachkern' are distinguishable at an early stage of development, but do not reach a functional condition until the young attains the free life, — a relatively much later stage of development than that at which it becomes functional in ganoids and teleosts. The habenular (olfactory) constituents of Reissner's fibre are developed and probably functional before the optic elements arise.

The ependymal groove, hitherto unnoticed in selachians, is highly developed and forms a conspicuous structure, in Raja extending up into the narrow recess above the posterior commissure. In Mustelus it has a greater lateral extent, and is curved upon itself so as to form a partially enclosed tube, horseshoe-shaped in transverse section. In Raja it still preserves in part the bilateral arrangement so conspicuous in cyclostomes.

The cells of the optic reflex apparatus constitute the so-called 'Dachkern,' a nidulus of giant cells lying in the tectum opticum immediately ventral to the dorsal decussation, on either side of the median plane and extending caudad to the cerebellum. They are more numerous and of relatively larger size in Raja than in Squalus or Mustelus. The cells are variable in form. Each cell is surrounded by a pericellular capsule of neuroglia and ependymal fibres. Fine nerve-fibres, probably from the dorsal decussation, end in arborizations over the body of the cell. The nucleus is always eccentric and opposite the largest process. The cytoplasm is finely granular, the Nissl's granules being most conspicuous near the periphery of the cell.

The cells are multipolar, and give rise ultimately to three principal processes. The smallest passes with the fibres of the dorsal decussation into the stratum medullare profundum, where they come in contact with the endings of the retinal fibres of the optic nerve. A second, coarser process, directed caudad, goes to form, on either side of the median plane, the tractus tecto-cerebellaris, which breaks up in the molecular layer of the cerebellum. A portion at least of these fibres decussate on entering the cerebellum. The third process, directed cephalad, forms with others of its kind the tractus tecto-fibrae Reissneris of either side, which, entering the ventricle through the characteristic ependyma at the anterior end of the optic lobes, fuses with other fascicles of fibres from the habenulae to form the fibre of Reissner. The exact method by which the fascicles enter the ventricle and unite to form Reissner's fibre differs in different species. The fibre attains its greatest observed diameter (25 micra) in some of the larger selachians.

Through the posterior portion of its course the fibre gives off numerous branches, which enter the walls of the cord. The posterior canal-cells are early developed, and send their axons cephalad to join Reissner's fibre. Each remains, by means of several dendrites, in connection with walls of the cord from which it is derived.

III. Ganoids.

A. Observations.

"The Ganoids are the most generalized of the true fishes, those nearest the stock from which the Teleosts on the one hand, and the Dipnoi and Batrachia on the other hand, have sprung" (Jordan and Evermann, '**96**-**00**, p. 98). Therefore this group offers a most interesting and fertile field of investigation to the student of comparative neurology. The brain is of a simple generalized type, perhaps nearer to the direct line of brain development than that of any other group. I have been favored in my studies in this group by a full series of larval Amia and adult brains of several other species. The only mention of this apparatus in the literature of the group is the brief description of its cells in Acipenser contained in the papers of Goronowitsch and of Johnston, which will be referred to later, and a brief reference to the description of Goronowitsch in a paper by C. L. Herrick.

1. Amia calva. *a. Development.* It was in the study of the development of Reissner's fibre in Amia that the cellular connections of the fibre were first made out, and the elaborate apparatus of which it forms a part discovered. For this work Amia is the most favorable material I have yet encountered, as the cells are large and the development of the apparatus takes place almost entirely after hatching. Moreover, the detailed studies of Reighard (:**03**) on the development of functional activities in larval Amia make it possible to correlate the development of structure and function. The development of the optic reflex apparatus has been completely followed in a series of larvae of Amia from the time of hatching to thirty days after. Some fifteen stages, cut in transverse, sagittal, and frontal sections, covering this period have been

studied. This material was preserved for me in Zenker's fluid, and has been stained for the most part with iron hematoxylin.

In the constriction between the second and third primitive brain vesicles (Plate **4**, Fig. 23), which later become respectively the diencephalon and mesencephalon, there is just before hatching a differentiation in the dorsal portion of the constricted nerve-tube. The dorsal wall thickens rapidly, and the flexure becomes more accentuated, growing downward and forward and further constricting the passage between diacoele and mesocoele. In this thickening there arises during the first day [1] of larval development a partial constriction, dividing it into two unequal portions (Fig. *B*). From the smaller anterior portion, belonging to the diencephalic roof, is formed the epiphysis (Plate **11**, Fig. 71). The posterior thickened portion develops rapidly, finally becoming sharply flexed as it grows ventrally and posteriorly. It is through this downward flexure of the roof that the posterior commissure develops about the third day (Plate **4**, Fig. 24, *com's. p.*).

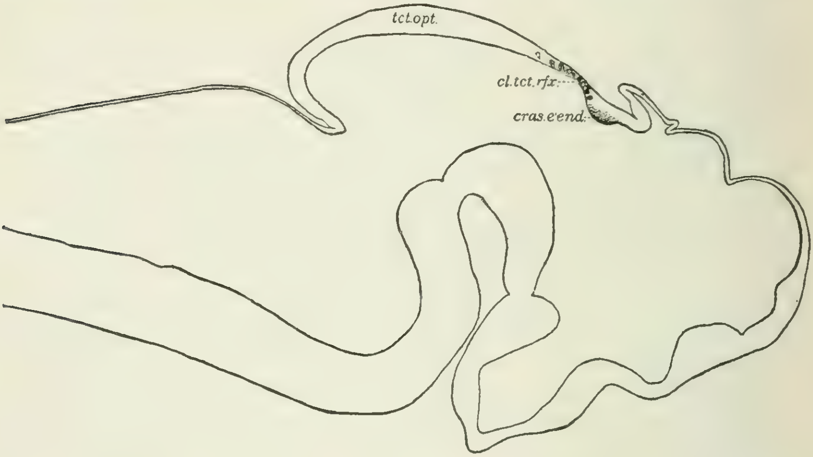

FIGURE *B*. Amia calva, larva of first day. Sagittal section of the brain, showing the position of the tectal reflex cells, and the great size of the encephalic cavities. For meaning of abbreviations, see Explanation of Plates, p. 257.

In this thickened fold are developed at an early stage numerous ependymal cells, from which the ependymal groove is formed. Running from the upper to the lower margin of the fold, they give a somewhat radiated appearance to this region because of the sharp flexure of the wall. This region of the brain thus has early in development a characteristic appearance (Plate 4, Figs. 24, 25), which is remarkably constant throughout life in all the lower vertebrates.

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[1] The age here given is from the time of hatching, which according to Reighard (:03) and Dean ('96) corresponds usually with the eighth to tenth day after fertilization.

(1) *Optic Reflex Cells.* The first differentiation in the neuroblasts takes place in Amia just before hatching, in the anterior portion of the roof of the third ventricle. In the region which later becomes the anterior part of the optic tectum, some of the neuroblasts have increased in size, have become more spherical in form and more definite in outline, and take stains somewhat more deeply than the surrounding neuroblasts (Plate 4, Fig. 23, *cl. opt. rfx.*). These cells are concentrated for the most part on either side of the median plane. A few scattering cells extend posteriorly and laterally through the tectum. There are from twenty to thirty of these cells at their first appearance, but they rapidly increase in number by the differentiation of additional cells.

In Amia the first day after hatching the number of cells is from 30 to 60. They are spherical and from 9 to 14 micra in diameter, thus being conspicuous among the surrounding neuroblasts (Fig. 24, *n'bl.*), whose outlines are less definite and whose diameters are less than half as great. The tectum in the median plane is so thin at this stage that a single cell often occupies its whole thickness. The protoplasm of the cell stains deeply, and the nucleus is large and clear, its diameter being somewhat more than half that of the cell. The single nucleolus stains deeply and is about two micra in diameter. The nucleus is central, not eccentric as in later stages. During the first day the axons develop from the cells as extremely fine processes. They first appear always on the side of the cell nearest the ventricle, growing directly toward it (Fig. 24, *ax.*). About the end of the first, or the beginning of the second day, these axons penetrate into the ventricle, passing between the epithelial or ependymal cells which line the roof of the ventricle.

In larvae from two to three days after hatching, the cells have increased in size and number. The number, somewhat variable at all stages (as shown in the table, p. 185), is now from 60 to 100. The average cell has now a diameter of 14 micra, two or three times that of the surrounding neuroblasts. The nucleus is large, 8 micra in diameter, and eccentrically placed opposite the side of the cell from which the axis-cylinder emerges. The tectum at its anterior end is still so thin that the cells lie in a single layer. The axis-cylinder, a delicate fibril, comes off from the almost spherical cell on the side nearest the third ventricle; it passes directly toward the ventricle, into which it projects, growing into the cerebro-spinal fluid. During the second day of larval development the axons may be seen projecting into the ventricle from the anterior portion of the optic tectum and torus, resembling cilia in their length and appearance.

Early in the third day the adjacent axons, projecting into the mesocoele, come together in groups of three to six, and begin to coalesce, forming apparently a single fibre. At a later stage in the third day the fibres formed by these united axons have grown farther into the ventricle, converging and coalescing as they grow backwards, and thus form the fibre of Reissner (Plate **4**, Fig. 25). At first these elements of Reissner's fibre are but loosely aggregated, often appearing as a confused, tangled mass of fine fibrils, but the union becomes more complete as the fibre grows caudad and development proceeds.

In Amia of the fourth day the region of the optic lobes has advanced rapidly in development and attained the condition typical of larval life. The posterior commissure is well established, and its development is already producing a downward growth in the median plane (Plate **4**, Fig. 25, *co'ms.p.*). The tectum opticum has attained greater thickness and the cells are now more closely aggregated at its anterior end in the median plane, but extend posteriorly rather irregularly and at greater intervals through the tectum on either side of the median plane, showing some tendency posteriorly to group themselves in 3's and 4's. The cells have increased slightly in size, but there has been no further increase in number. The system of axons in the optic ventricle is still diffuse at the anterior end, and even as far back as the cerebellum, but more compact posteriorly. Each axon emerges into the ventricle separately, most of them in or near the median plane, the system showing to best advantage in median sagittal sections. As the tectum has developed in thickness by the multiplication of surrounding neuroblasts, many of the cells have been withdrawn, so to speak, from immediate proximity to the ventricle by the development of intervening tissue, and their axons have taken a course through the tectum toward the recess above the posterior commissure, where near the median plane the axons emerge into the ventricle (Plate **5**, Figs. 36, 37).

By the sixth or seventh day (Fig. 35), this whole apparatus has attained the condition typical of larval life, and later in the adult undergoes only slight modification. In the development of the tectum opticum and posterior commissure, the position of the cells and their relation to the optic ventricle have become considerably changed, and consequently the course of the axons before entering the ventricle has become modified and complicated.

The cells, 70 to 100 in number, but showing in this respect, variation from individual to individual, are for the most part aggregated in or near the median plane, dorsal and posterior to the posterior commissure, and are grouped about the anterior and dorsal walls of the mesencephalic recess, which extends above the posterior commissure (Plate **5**, Figs. 37, 38). From this region the cells extend caudad and laterad into the optic tectum on either side of the median plane. Cephalad the cells are closely aggregated (Figs. 34, 38), but caudad they are irregularly distributed, a few stray cells sometimes occurring far out in the tectum (Fig. 36).

In the earlier stages, where all the cells are in immediate proximity to the ventricle, the axons pass directly toward the ventricle and into it, (Plate **4**, Fig. 25). In the later stages the cells, in addition to having undergone a seeming migration, have in many cases become partially rotated by the development and consequent pressure of intervening cells. Anteriad, where the cells are closely aggregated, those cells which lie near the median plane have undergone a rotation of 90 degrees, so that their axons now pass first laterad, and then caudad, describing a curve and entering the ventricle near the median plane (Plate **4**, Fig. 26). In these cells, as seen in cross-sections of this region, the nucleus lies with great uniformity at the mesal end of the cell, so that cells which lie close together, but send their axons in opposite directions, have their nuclei in juxtaposition (Plate **5**, Figs. 36, 37, 38). The cells which lie well caudad and laterad in the tectum send their fibres cephalad (Plate **4**, Fig. 26; Plate **5**, Fig. 33), forming on either side of the median plane loose fibre tracts which curve mesad toward the torus, the axons entering the ventricle near the median plane. The cells which still lie near the ventricle send their axons directly into it (Plate **5**, Figs. 33, 35). In all these changes of the positions of the cells, the nucleus uniformly retains its position at the end of the cell opposite to that from which the axon emerges.

The axons running proximally toward the ventricle pass between the epithelial cells lining its surface and into the ventricle, and then straight onward for a short distance; then they are deflected toward the median plane and coalesce with adjacent axons, eventually forming Reissner's fibre (Plate **4**, Fig. 26, *fas. Reis.*) by the union of all the axons of the 80 or 90 cells.

The union of the axons in larvae of six days and more occurs relatively nearer the point where the axons emerge than in younger specimens, the coalescence and consolidation becoming more complete with the advance in development. Compare Figures 25 (Plate **4**) and 35 (Plate **5**).

Near the median plane the axons run straight into the ventricle, converging, with gradual coalescence, toward the fibre. Such axons as emerge into the ventricle further from the median plane run out a little distance from the wall and roof of the ventricle, then turning at right angles unite, converging toward the anterior end of the ventricle, where the united bundles curve backward and join with those of the median plane and of the opposite side to form Reissner's fibre.

The cells at this stage are still conspicuous and of large size, compared with the surrounding cells, having a diameter of from 12 to 20 micra. From the cell arise, in addition to the axon described, a number of other processes. These could be followed only a short distance in Amia at this stage. Some of them, however, run posteriad toward the ectal region of the optic tectum.

In the eight-day Amia, Reissner's fibre has become still more consolidated at the anterior end, that is, the coalescence of the axons has continued to progress anteriad (Plate **5**, Figs. 32, 33, 34). In this stage some of the axons coalesce before emerging into the ventricle. This was shown by carefully counting the number of cells (71), and then counting the separate axons at the point where they enter the ventricle (55). This counting could be done with some degree of accuracy, and showed that there had been union within the tectum. Counting now the separate fibres crossing an imaginary curved surface located in the ventricle, and everywhere 0.1 mm. from its roof and walls, the number is 25, and still nearer the median plane of the ventricle the number is reduced to six or seven.

In the seventeen-day stage the constituent elements of Reissner's fibre entering the ventricle have undergone further coalescence. The anterior end of the fibre, however, still has a dendritic form (Plate **5**, Fig. 35), owing to the many separate bundles of axons which enter the ventricle. These unite into several main trunks, which run nearly parallel through the anterior portion of the mesocoele before coalescing. From the middle portion of the mesocoele, caudad, the union is complete. In the thirty-day stage there are only six or eight chief divisions of the fibre in the anterior portion of the mesocoele, and in the adult this number is still further reduced.

Following is a tabulated record of the number and size of the optic reflex cells of larval Amia, at different stages:

Stage.	No. of Cells.	Diameter in micra.								
		Maximum.			Minimum.			Average.		
		Cell.	Nucleus.	Nucleolus.	Cell.	Nucleus.	Nucleolus.	Cell.	Nucleus.	Nucleolus.
1 day	38 29 59	14	9	2+	9	7	2	12	8	2
2 days	41 59	14	9	3−	9	5	1.5			
3 "	94 76	17	8	3−				15	7	2
4 "	86 84									
5 "	84 43+	13	7	2						
6 "	65+									
8 "	83 71 69	20	9	2				15	9	2.5
17 "	53+	25	12	3	10	6	2	17	8	2.5

(2) *Posterior Canal-Cells.* By the third day of larval development in Amia, Reissner's fibre has grown backward through the fourth ventricle and the whole length of the central canal. In earlier stages careful search fails to show any trace of the fibre, except at the extreme posterior end of the canal. Here I early saw a delicate fibre, but could not trace it forward to the middle and anterior portion of the canal. This was the cause of much perplexity until I discovered at the extreme end of the canal the cells from which it arose.

At this stage the posterior end of the cord (Plate 4, Fig. 31) is slightly enlarged at its tip, and within this enlargement the canal dilates to form the ventriculus terminalis. There is no exterior opening in this ventricle, such as the sinus which has been described in Petromyzon by Studnicka ('95), nor is there evidence of a persistent remnant of the neurenteric canal, such as I have figured in Squalus (Plate 3, Fig. 22).

At the time of hatching one may find in the extreme end of the canalis centralis a number of small cells, 3 to 4 micra in diameter, lying in the lumen of the canal and in the ventriculus terminalis (Plate 4, Fig. 27). The walls of the cord surrounding this part of the lumen are made up of a single layer of loosely aggregated cells, some of them in process of division (*cl. g.*, Plate **4**, Fig. 27, Plate **5**, Fig. 39). Certain of these cells become separated from the wall of the canal and pushed into the lumen.[1] I have seen such cells still an integral part of the wall but projecting into the canal. In Figure 30, Plate **4**, two cells (α, α) are shown lying close to the wall, from which they have been given off and with which they are still connected by protoplasmic strands. These two cells are in contact with each other, and are apparently connected with a third (γ) by a protoplasmic bridge. In the same figure the two small cells (β) in the same region are perhaps to be thrown off later, or they may represent the residual halves of the mother cells from which α, α were formed. Such cells usually continue for a considerable period to lie close to the portion of the wall from which they were given off, and probably always maintain their connection with it (Plate **4**, Fig. 29). In Figure 27 are shown two small cells (α) lying at the side of the ventriculus terminalis, close to one of the actively dividing germinal cells, 'Keimzellen' of His (**'89**), from which they may have been derived. In the upper portion of the figure are several dorsal cells ('Hinterzellen') in an early stage of development, some of which are being pushed out from the cord. Of these cells in the canal and terminal ventricle, many atrophy and disintegrate. Some eight to twelve persist and continue to develop. These, at first spherical in form, become somewhat spindle-shaped as they increase in size (Plate **5**, Fig. 39, *cl. can. p.*).

The axon is given off from the more tapering anterior end of the cell and runs forward through the lumen of the canal. The developing axon is exceedingly delicate, and cannot be followed to its tip, as it becomes finer and finer till it fades away (Plate **4**, Fig. 31, α). From what I have seen I believe the process of growth of the axon, as in the cells of the tectum, must be similar to the streaming movement of the pseudopodia in certain Heliozoa, and not at all like the growth of a root-tip. At first the axons of these cells are quite separate, but eventually they coalesce in much the same manner as do the axons of the tectal reflex cells. Two or more axons may unite to form a single fibril, which in turn uniting with similar fibrils forms the main trunk running cephalad (Fig. 31). These separate fibrils may run parallel for some distance before uniting. This I found especially true in the catfish (Plate **8**, Fig. 61, *ax.*), where as many as four such fibrils may be found in one cross-section. This perhaps offers an explanation of Studnicka's statement, that there are sometimes two or three parallel Reissner's fibres. The manner in which these axons unite to form a single trunk varies with different species and is characteristic of them; it will later come up for further treatment. In Amia the more usual method is for the axons to unite singly with the main trunk. In later development they become more closely consolidated with Reissner's fibre, appearing as fibrils coming from it (Plate 4, Fig. 31).

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[1] A similar process takes place normally in the central canal during larval life at least. Cells from the walls of the canal are pushed out into the lumen, where they may be found in various stages of disintegration (see footnote, p. 143).

As the cell develops, the protoplasmic strands which connect it with the wall of the cord (Plate 4, Figs. 27, 30) become metamorphosed into dendrites, and at the same time numerous other branching dendrites arise and, growing out through the fluid of the canal in various directions, penetrate the cord on all sides of the canal (Plate 4, Figs. 28, 29). Often a large process resembling an axon runs caudad through the canal. After passing into the tissue of the cord these dendrites could be traced no further. The cells which make up the walls of the cord at this early stage have no cell walls that can be demonstrated, and the dendrites are lost in the apparently homogeneous protoplasm filling the spaces between the large nuclei (Figs. 28, 29).

The nucleus of these posterior canal-cells is always large and sharply defined. The chromatin is variously arranged, but usually, especially in later stages, forms a more or less irregular network. As a rule a large spherical nucleolus is present. The cytoplasm of the cells in their early stages is somewhat diffuse and stains very lightly (Fig. 27, *cl. can. p.*). Later it becomes denser, and the cells take a more definite outline and stain sharply (Fig. 28).

By the sixth day of larval life these posterior canal-cells have increased in size so that one of the larger of them, having a diameter of 8 to 10 micra, nearly fills the lumen of the canal. In Figure 28 (Plate 4) such a cell is shown lying in the ventriculus terminalis, 30 micra (three sections) from the end of the lumen and 60 micra from the extreme end of the cord. Another such cell, lying just anterior to it, and distant about 30 micra, is shown in Figure 29. These multipolar cells send their processes peripherad about equally in all directions.

During the third and fourth day of larval life the system of axons of the posterior canal-cells growing cephalad, now more or less consolidated, meets, in the posterior third of the canal, the system of axons from the tectal cells growing caudad, and by some process of interlacing or intercalating not yet clearly made out, the two systems unite to form the so-called Reissner's fibre (Plate 4, Fig. 31, *fbr. Reis.*; Fig. *M*).

Somewhat later Reissner's fibre develops a thin medullary sheath of myelin throughout its length. As there is no trace of either Henle's or Schwann's sheath, the myelin must necessarily be a secretion of the axis-cylinders. If any medullary sheath is developed about the separate axons where they are free, it is so slight that it has not yet been distinguished.

It is probable that the axons of the tectal reflex cells running caudad have entered the cord before Reissner's fibre has become completely consolidated and developed its medullary sheath. Numerous appearances in my sections lead me to think so. But it is not until the eighth day in Amia that I have seen what I could positively identify as axons entering the cord. They then come off as delicate branches of Reissner's fibre, and pass through the canal obliquely (usually making an angle of about 45° with the fibre) and enter the cord from the ventral portion of the canal. They can frequently be traced passing between the ependymal cells which line the walls of the canal, and for a short distance into the cord, where they are lost. I believe there is no doubt of their connection with the ventral root and musculature, evidence of which of another kind is given elsewhere in this paper. I have no knowledge, however, as to whether they pass out of the ventral root directly to the musculature, or whether they connect with another neuron. The branches from Reissner's fibre are supposedly the axons of the tectal cells, though as to their course through Reissner's fibre, I only know that it shows a fibrillar structure.

b. Adult. From the 30-day larva, 2 cm. long, to the adult, I have studied no intermediate stages. Although there is a great development in the mesencephalon as a whole, the optic reflex apparatus remains *in statu quo*, except as the development of the optic lobes affects its relations. Comparing the adult Amia (Fig. *D*) with the 30-day larva (Fig. *C*), we see that the tectum has increased greatly in thickness, owing to the development of the cellular elements; but near the median plane it is due more particularly to the growth of the optic tracts. The tectum has grown so as to bulge cephalad and dorsad from the posterior commissure, consequently increasing the size of the mesocoelic recess in the median plane above the posterior commissure. At the same time the posterior commissure has developed posteriorly between the optic reflex cells and the ependymal thickening (Figs. *C*, *D*), so that the ependymal thickening has apparently receded cephalad. As a result of these morphological changes, the optic reflex cells have come to lie on the anterior and lateral walls of the recess. It is into this recess that the axons and fibrils formed from the coalesced axons of the optic reflex cells emerge, and joining within it form Reissner's fibre (Plate 6, Fig. 40, *rec. ms'coel.*).

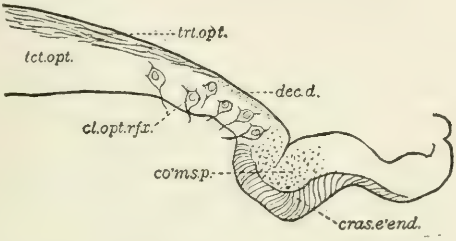

FIGURE *C*. Amia calva, larva of thirty days. Diagrammatic sagittal section of the anterior portion of the midbrain roof. × 87. For meaning of abbreviations, see Explanation of Plates, p. 257.

The cells in the adult have an average diameter of 18 micra, about the same as in the larval stages, and are consequently much smaller than in Polypterus. In addition to the axon, there are two principal neurites given off from the cells and a number of finer dendrites. These two neurites can sometimes be traced for a considerable distance. They proceed dorsad and laterad through the middle of the vertical portion of the

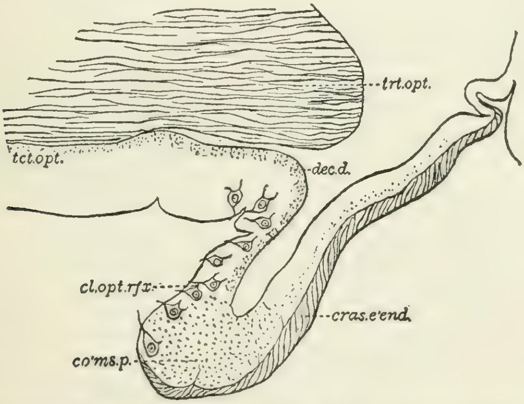

FIGURE *D*. Amia calva, adult. Diagrammatic sagittal section of the same region as in Figure *C*. × 41. A comparison of the two figures illustrates the morphological changes affecting the position of the tectal reflex cells and the surrounding brain structures in the transition from larval to adult conditions. For meaning of abbreviations, see Explanation of Plates, p. 257.

tectum (Fig. 40, *ax.'*) and, passing laterad to the flexure in the tectum, run caudad. A tract which I believe is formed from one set of these neurites has been traced, somewhat discontinuously, through the lateral part of the tectum into the cerebellum. The other set of neurites probably runs to the middle layer of the tectum, as traced in other forms.

2. Lepidosteus osseus. The material of this species at my disposal has been limited to young larvae from 1 to 2 cm. long. The optic reflex apparatus is in its development and arrangement in all essential respects similar to that of Amia. Some differences, however, are to be noted. At the time of hatching, the development of the apparatus is somewhat farther advanced than in Amia, though the nervous system as a whole is less developed. As in Amia, the cells lie at the anterior end of the tectum, but are less numerous and smaller. In larvae 15 cm. long they are about 10 micra in diameter. The cells are multipolar (Plate 6, Fig. 44), giving off usually two principal processes in addition to the axon, which runs obliquely ventrad and cephalad and emerges into the ventricle in the median plane in the region just posterior to where the posterior commissure later develops. The axons, after passing into the ventricle, are deflected obliquely backward and anastomosing form a plexus from which Reissner's fibre develops (Fig. 44, *ax. cl. tct. rfx.*).

Reissner's fibre is a little greater in diameter than in Amia of the same size (1.2 micra as against 1 micron in Amia larvae 15 mm. long). The branches from the fibre which later enter the walls of the cord in its posterior portion are also correspondingly larger, so that Lepidosteus is favorable material in which to study these structures. They run off from the fibre obliquely caudad, and for the most part enter the cord at the ventral sides of the canal (Plate 6, Fig. 45). These branches probably represent the individual axons which make up Reissner's fibre. They pass between the cells of which the wall of the cord is composed, and are lost.

The posterior canal-cells (Fig. 46) are present as in Amia, but probably fewer in number. I have found them only in the ventriculus terminalis. They are multipolar and send off their processes to all sides of the canal and into the cord.

3. Polypterus bichir. My studies on this species are based on a number of adult brains taken and prepared by the late Dr. N. R. Harrington while on the Senff Zoölogical Expedition to the Upper Nile. The marked peculiarity of this species is that the cells of the optic reflex apparatus lie not only dorsal and posterior to the posterior commissure, as is normal in other forms, but also ventral and anterior to it, so

that in the median plane they almost surround the border of the commissure (Plate 6, Fig. 41). Comparing the condition in adult Amia (Fig. 40) with this, we see that there the cells lie about the border of the recess of the mesencephalon, which extends forward above the posterior commissure, but they are always morphologically posterior and dorsal to the commissure. In Figure 40 one cell (a) is shown which might be considered as ventral to the commissure. This mesencephalic recess is not much developed in Polypterus.

The explanation of this position of the cells in Polypterus is probably embryological. In the development of the commissure the nerve-fibres, growing across from the opposite sides, have passed above and among the already established cells, instead of anterior to them, as in other ganoids and indeed in most vertebrates. As a result of this condition many of the cells are crowded out of the median plane and lie laterally in the tectum about the anterior border of the ventricle (Plate 6, Fig. 42, *cl. tct. rfx.*).

The cells are multipolar, having two principal processes in addition to the axon. The axon of the cell is frequently found projecting into the ventricle (Fig. 42, *ax.'''*). Usually, however, it takes a less direct course through the brain tissue toward the median plane, uniting with other axons before emerging. The more prominent of the other two processes (Fig. 42, *ax.''*) has been traced into the middle layer of the tectum. The third process has been followed only a short distance. The cells are much larger than in Amia, having an average length of 45 micra and breadth of 30 micra.

4. ACIPENSER. I have had no opportunity to study Acipenser, having no material at my disposal, but through the courtesy of Dr. J. B. Johnston I have been permitted to examine his sections. In general the conditions are much as in Amia and Polypterus, except that the cells, as might be expected in a fish of such great size, are much larger.

Goronowitsch ('88) was the first to observe the optic reflex cells in ganoids. I quote from his description of the brain of Acipenser ruthenus (p. 553). "In den proximalsten Abschnitten des Tectum wird die Körnerschicht dünner. Medial von derselben erscheinen sehr grosse, ziemlich dicht zerstreute Ganglienzellen. Die feinen Fortsätze dieser Zellen verlaufen radial zur Oberfläche des Tectum, es schien mir dabei, dass sie mit Opticus Fasern in Verbindung treten, welches Verhalten aber ohne zweckmässige, speciell darauf gerichtete histologische Untersuchung nicht festgestellt werden kann." He believed these cells to be homologous with the cells of the 'Dachkern' described by Rohon in selachians.

Johnston (:**01**, p. 50), in his magnificent monograph on the brain of Acipenser rubicundus, has described this group of cells, under the name of 'nucleus magnocellularis,'[1] as located "in the cephalic part of the tectum, at either side of the mid-dorsal line." "These cells are never impregnated in Golgi preparations, so that it is impossible to study their dendrites satisfactorily, or to determine whether they have neurites. A few of the cells are found as far caudally as the middle of the tectum, but the great majority are situated dorsal to the posterior commissure, surrounding a blind pouch of the aqueduct which extends forward above the commissure. The cell body is usually pear-shaped with a single large process from the smaller end. There may be two or more smaller processes from other parts of the cell. The smaller end of the cell is usually turned away from the cavity and the large process arising from this end of the cell turns at once laterally and can be traced for some distance in hematoxylin sections as a very thick fibre running just over the ependyma cells and among the nerve cells of the inner zone."

B. CRITICAL DISCUSSION.

The large process described by Johnston in Acipenser is probably the axon of the cell which goes to form Reissner's fibre, and in young larvae would doubtless have been found passing directly into the ventricle; but in the adult state, as in other ganoids, it takes a tortuous path before emerging into the ventricle in the median plane. As Reissner's fibre was not observed, and only adult brains were studied, it is not surprising that Johnston failed to recognize the true significance of these cells.

Though Johnston (:**01**, pp. 72, 73) was unable to follow the processes of the cells of the nucleus magnocellularis, he describes a tract of fibres, which were impregnated in his preparations, running from this region to the cerebellum. This "tractus tecto-cerebellaris I" consists of coarse fibres and "arises from the cephalic part of the tectum." "I have been unable," he adds, "to find its cells of origin. The greater number of the fibres arise from the vicinity of the large-celled nucleus. I have traced the fibres down among the cells, but there are no cells impregnated when the fibres are stained. Other fibres join this bundle from the cephalic border of the decussation. The fibres, which are relatively coarse and are beautifully impregnated in many preparations, form a compact bundle which courses around the lateral border of the tectum in the bottom of the shallow groove which limits the tectum laterally. As it proceeds it is augmented by occasional fibres from the lateral part of the tectum. When the bundle reaches the point of junction of the tectum and cerebellum, it enters the cerebellum and is distributed to the body and valvula. As the bundle enters the cerebellum the fibres grow distinctly larger, then divide into two nearly equal branches, each of which further increases in size and becomes rough and irregular like a dendrite. One branch of each fibre ends in the valvula, and these have no regular arrangement. The other branch takes a straight course with its fellows in a bundle along the lateral surface of the body, just ventral to the line of connection with the lateral lobes. They break up in end branches in the lateral portion (granular layer) of the body, although a few fibres reach the keel (molecular layer) at the caudal end of the body."

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[1] Johnston is the first to apply the term 'nucleus magnocellularis' to this group of cells, though Edinger (:**01**) almost simultaneously used the term 'nucleus magnocellularis tecti' in connection with the homologous group of cells in Scyllium. Though these terms, particularly in the latter form, describe this nucleus adequately for the animals studied by Johnston and Edinger, it is not equally applicable to other groups, such as the teleosts and mammals, where the cells are not of unusual size. Moreover, these terms are liable to confusion with similar terms long used in connection with other cell groups. Reissner described a 'nucleus magnus' lying in the posterior part of the optic lobes (see Bellonci, '**88**, p. 26; and Haller, :**00**, p. 298). Stieda described the 'nucleus magnus thalami.' A 'nucleus magnocellularis diffusus' has been described in the medulla, ventrad to the posterior end of the 4th ventricle (see Rabl-Rückhard, '**94**, p. 711; also Kölliker, '**96**, pp. 323, 325).

By the aid of Johnston's description I have been able to follow this tract in my hematoxylin preparations of Amia, though not continuously. It is, I believe, derived from the third process of the cells of the nucleus magnocellularis, and is homologous with the tecto-cerebellar tract which I have described in selachians and birds. It establishes connection between the optic reflex apparatus and the cerebellum, by which the coordination of muscular movements is brought about.

The fact that the tract in Acipenser "courses around the lateral border of the tectum," while in Raja it passes straight back through the roof of the tectum just lateral to the median plane, does not militate against this homology, as the terminals are the same, and in each case the shortest path is followed (see Johnston's Figure 4). Moreover, the change in the relative position of this tract is easily understood in taking into account the great lateral development of the optic lobes in Raja.

Johnston has described in Acipenser certain small fusiform cells, which were impregnated in his preparations, lying near the median plane and bordering on the ventricle. He designates these as, — "Type C: cells of the torus longitudinalis Halleri," and believes them homologous with the cells of the torus described by Sala.[1] This conclusion must be set aside, as the disposition of the axons and dendrites of these two classes of cells is not identical, and moreover I believe I have conclusively shown that it is the cells of the 'nucleus magnocellularis' of Johnston, lying in the same region, which are homologous with Sala's torus cells.

C. Summary for Ganoids.

The ganoid brain is relatively simple in structure and presents a rather generalized type. It is, therefore, an excellent starting-point for almost any investigation in comparative vertebrate neurology, and has been found especially so in the study of the endings of the optic reflex apparatus.

The apparatus is late in development in ganoids, and is not fully established until several days after hatching. The cells, 40 to 100 in number, lie close to the ventricle in the anterior portion of the tectum opticum in, or near, the median plane, and for the most part are grouped about the margin of that portion of the ventricle which extends above the posterior commissure. At an early stage of larval development these cells send their axons separately and directly into the ventricle of the optic lobes. The axons, growing caudad through the fluid-filled ventricle, come together and coalesce, forming Reissner's fibre. In later development this coalescence progresses anteriorly, even into the tectum, so that the number of divisions of Reissner's fibre in the ventricle is greatly reduced. The fibres of the posterior commissure, developing after the axons of the optic reflex cells have emerged into the ventricle, usually take a course anterior and ventral to the cells, but in Polypterus some of the cells are ventral to the commissure.

In adult ganoids, the development of the dorsal decussation of the tectum across the thin median roof of the mesencephalon has crowded downward the large cells of the optic reflex apparatus ('nucleus magnocellularis' of Johnston) so that they form two elongated prominences separated by the median fissure. These ridges, the homologues of the lobes of the torus longitudinalis of the teleost brain, do not appear in the larval ganoid brain, and in adult Amia are scarcely distinguishable.

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[1] There is no authority for the application 'Halleri,' as the term torus longitudinalis was first used by Stieda ('68, pp. 24 *et seq.*).

In Acipenser they are but slightly developed, but have been noticed by Johnston (:01, Fig. 20). In adult Lepidosteus they are somewhat more pronounced and were observed and figured by C. L. Herrick ('91).

The cells are multipolar, giving off two principal processes in addition to the axon. One of these processes has been traced dorsad and caudad near the median plane into the middle fibre layer of the tectum, whence it probably bends ectad and comes into contact with the afferent nerve-fibres of the optic tracts. The optic tracts coming from the thalamus enter the tectum at its anterior border (Plate 6, Figs. 40–42, *trt. opt.*), and run posteriad through the ectal portion of the tectum near the median plane, giving off their fibres in bundles to break up in the outer third of the tectum. The second process of the optic reflex cells passes ectad into the superficial portion of the tectum and enters a tract running through the lateral wall of the tectum and into the cerebellum, thus establishing connection for the co-ordination of the muscular movements to which the apparatus gives rise.

Through the distal portion of the canalis centralis Reissner's fibre breaks up into its constituent axons, which pass into the ventral part of the cord, and probably out through the ventral roots to the musculature. In the extreme posterior portion of the canal, and in the ventriculus terminalis, there are from five to ten posterior canal-cells, derived from the walls of the cord, which send their axons cephalad through the canal and joining Reissner's fibre become a part of it. These cells are in intimate connection with the cord by numerous processes.

IV. Teleosts.

The teleost brain presents a form of great complexity, considerably removed from the more generalized types found in ganoids, selachians, and amphibians. This large and variable group presents many types of brain structure, and in common with this the morphology of the mesencephalon varies greatly. Consequently, the optic reflex apparatus undergoes considerable modification in the nature of its constituents and in its relations to the surrounding brain structures. This makes a broad and comprehensive study of the apparatus in this group desirable, and with this in view I have examined "all the fish that came to my net," representing thirty families and about sixty species.

A. Historical.

Though the nervous system of teleosts has received so much attention from comparative neurologists, Reissner's fibre has been noticed by only four investigators, Stieda, Sanders, Mayser, and Studnicka. Stieda ('**68**, p. 11) described it as occurring in the central canal of all teleosts that he had examined (Gadus, Cyprinus, perch, shad, eel, and pike). In his Taf. 1, Fig. 9, he has shown it within the canal in a transverse section of the cord of the pike, and in Fig. 12 in a longitudinal section of the cord of Gadus lota. Sanders ('**78**, p. 742) described a rod within the canal, which he found occasionally in sections of the cord of the mullet (Mugil).

Mayser ('**82**, p. 295) found Reissner's fibre in the fourth ventricle in cyprinoids, and traced it forward to the region of the trigeminal nerve. In his Figuren 20 und 21 he has shown it in cross-sections of the cord lying within the central canal. Studnicka ('**99**, p. 3) merely mentions its occurrence in Anguilla.

Stieda and Sanders believed the rod which they saw within the canal to be an artifact formed by the action of the fixing fluids on the contents of the canal. Mayser and Studnicka looked upon Reissner's fibre as a preformed structure existing during the life of the animal.

Bellonci ('**81**), Fusari ('**87**), and C. L. Herrick ('**91**) observed the fibre-tracts from the torus to the tectum; and Sala ('**95**), P. Cajal ('**99**), and Catois (:**01**) studied the cells of the torus, without, however, suspecting their true relations or function. The only other references to any portion of the optic reflex apparatus in the literature of the teleost brain are in my previous papers (Sargent, :**00**, :**00**^{a}, :**01**, :**01**^{a}).

B. Morphology of the Mesencephalon.

1. The Median Zone. Burckhardt ('**94**) pointed out that the median longitudinal zone of the brain retained to a greater degree than any other portion, the primitive characteristics of the neural tube, remaining fairly constant, both in ontogeny and phylogeny, throughout the vertebrate series. The median zone is essentially ependymal, while the bordering lateral zones are highly nervous.

That portion of the median roof lying anterior to the posterior commissure, and between it and the base of the epiphysis, the pars intercalatus, designated by Burckhardt as the "Schaltstück," is in teleosts in an especially primitive condition (Fig. *I*). It is of considerable lateral breadth, and is for the most part made up of the thickened ependyma of this region (Plate **7**, Figs. 47–52, *cras. e'end.*), consisting of elongated, cylindrical, ependymal cells inclined obliquely ventrad and caudad. These constitute an inverted trough, or groove. This ependymal groove (Figs. *E*, *F*) is, however, not so prominent as in the selachians, but resembles more closely the condition in ganoids. The median zone of the mesencephalon, however, is probably more complex in some teleosts than in any other group. This is due to the development of the dorsal decussation of the tectum opticum and the crowding into the median plane of structures derived from the lateral zones.

2. THE TORUS LONGITUDINALIS. In the adult teleost brain the roof of the mesencephalon is complicated by the presence of the torus longitudinalis. In the adult this is typically a medianly grooved ridge extending downward from the thin median portion of the mesencephalic roof (Fig. *H;* Plate 8, Fig. 58). It usually extends from the posterior commissure through the length of the optic lobes, but is best developed at the anterior end. The relative size of the torus, and consequently its relations to the surrounding structures (tectum, posterior commissure, valvula cerebelli, etc.), vary greatly in the different groups of teleosts (see Sargent, :03^b).

Rabl-Rückhard ('87) first pointed out that each half of the torus is developed from the tectum of the corresponding side. The torus first appears ontogenetically, as a longitudinal thickening of the tectum on each side of the median plane, and becomes separated from the tectum by a longitudinal fissure. At first these longitudinal lobes are considerably separated, being connected by only the thin median roof of the mesencephalon. As differentiation advances and the lobes increase in size, this fissure is narrowed and in higher teleosts becomes obliterated. In the Siluridae the typical larval condition persists, the lobes remaining separated except at the extreme anterior end (Plate 9, Fig. 65).

The first differentiation of the torus lobes is at the anterior end of the tectum, and progresses caudad with advancing development. In nearly all teleosts the torus is early developed, and has attained its typical form at the time of hatching, but continues to increase in size up to the adult stage.

Phylogenetically the torus longitudinalis, as we have seen, has its beginning in the ganoids. With the increasing complexity of the adult ganoid brain, and the development of the dorsal decussation of the tectum, the cells of the optic reflex apparatus are crowded downward so as to form two longitudinal ridges, one on either side of the median plane, extending backward from the posterior commissure.

The Siluridae naturally supply the necessary connection in the phylogeny of the torus between the primitive condition in ganoids, and the highly differentiated state in the higher teleosts. In Amblyopsis

(Fig. *G*) the torus, as a result of arrested development, has ganoid characteristics.

The torus longitudinalis, then, is merely the mesal and primitive portion of the tectum constricted off, and as it were left behind in the enormous development of the tectum in this aberrant group. But though the torus is a structure which first attains an independent and definite form in the teleosts, and exists in that group only, its essential elements are perhaps the most archaic of the mesencephalic roof.

With this enormous development of the tectum opticum in teleosts, important morphological changes have been brought about. The optic lobes, carrying with them the torus longitudinalis, have grown cephalad, so that they project above and overlie the diencephalon (Fig. *I*; Plate **7**, Fig. 51). The posterior commissure, developing ontogenetically before this enormous anterior development has taken place, and being fixed in position, is not carried forward with the tectum. In the adult brain the posterior commissure, therefore, seems to have migrated caudad, and in median sagittal sections is seen projecting far backward from the anterior part of the tectum (Figs. 49, 51). A somewhat similar condition occurs in other vertebrates, but is not carried to nearly the same extent.

The torus longitudinalis curves around the margin of the recess above the posterior commissure, and at its anterior and ventral margin becomes fused with the posterior commissure (Plate **7**, Fig. 50; Plate **8**, Figs. 55–57). In the more highly differentiated and active teleosts the valvula cerebelli attains a great development and is crowded forward, largely filling the mesocoele (Fig. *I*). In these forms, as a result, the torus is crowded forward, so that it is shortened and comes to lie largely dorsal to the commissure (Plate **10**, Fig. 69); or it may be flattened along the roof, and in close contact with the valvula.

In the teleosts a greater or less portion of the torus lies dorsal to, and a considerable distance cephalad of, the posterior commissure. It thus comes about that the developing axons of these torus cells, in making their way into the ventricles, take the shortest path and enter the ventricle anterior to the posterior commissure. A somewhat similar condition was noted in Polypterus, where several of the optic reflex cells were found anterior to the posterior commissure. This phylogenetic change is explained by the ontogeny. The cells of the optic reflex apparatus are early developed, before hatching, and while the torus longitudinalis is as yet incompletely differentiated from the tectum. They send their axons into the ventricle from the median plane, of the roof (Plate **7**, Fig. 47; Plate **8**, Figs. 55, 56, 58, *fas. Reis.*), even before the posterior commissure has developed. In the later development the fibres of the posterior commissure, in making their way across from the opposite sides, pass posterior to these axons. Such a preposterior commissural tract, the tractus toro-fibrae Reissneris *anterior* (Figs. *I, J, K;* Plate **7**, Figs. 48, 51; Plate **8**, Figs. 56, 57, 59; Plate **10**, Fig. 69; *trt. tor. fbr. Reis. a.*) is found only in teleosts, though its beginning is foreshadowed in Polypterus (Plate **6**, Fig. 41).

C. The Optic Reflex Apparatus.

Siluridae (34).[1] In the Siluridae I have studied this apparatus in a series of larvae of Ameiurus nebulosus extending from the time of hatching to larvae 3 centimetres in length. The brain in the Siluridae is of relatively simple morphological structure, much the simplest of all teleosts. Its structure is transitional between the primitive type of the ganoidean brain and the aberrant complexity of the teleostean brain. The optic lobes are but little more developed than in the ganoids. The relations in the anterior part of the optic lobe are much the same as in Amia (Plate **7**, Fig. 47).

The torus longitudinalis is late in developing, and even in the adult is of small size and primitive structure, resembling the condition in ganoids. Each lobe appears as the median portion of the optic tectum slightly constricted off from the lateral portion by a shallow groove. Anteriorly the lobes of the torus converge and fuse in the median plane. Posteriorly they diverge till they are widely separated, being connected by only the thin median roof of the ventricle (Plate **9**, Fig. 65).

The optic reflex apparatus is already well established at the time of hatching and reaches full development soon after. In larvae 1 to 2 centimetres in length Reissner's fibre has been traced through the whole length of the central canal, and has a diameter of from 5 to 8 micra. Reissner's fibre runs directly cephalad through the optic ventricle to its anterior portion just behind the posterior commissure, where it breaks up into many branches. Numerous fine fibrils emerge from the anterior portion of the roof of the ventricle near the median plane and extend towards the fibre (Plate **7**, Fig. 47, *fas. Reis.*).

The optic reflex cells arise for the most part in the anterior end of the roof, and lie in the mesal portion of each half of the tectum, that portion which is later partly constricted off to form the longitudinal lateral lobe of the torus longitudinalis. During these early stages the sulcus between the halves of the tectum is of extraordinary width, *i. e.* the roof of the mesencephalon is very thin for a considerable distance on either side of the median plane (Plate **9**, Fig. 65).

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[1] The numbers given with the name of the family are those assigned to that family by Jordan and Evermann ('**96–00**), and will serve roughly to indicate the relationships of the several families.

The axons enter the ventricle from the mesal and anterior portions of the torus lobes immediately above the posterior commissure (Fig. 47). The cells of the torus show something of the arrangement, in rows radial to the mesocoele, characteristic of most teleosts. In the anterior part of the torus the cells are larger than posteriorly, perhaps because they are further advanced in development. They have in general a bipolar appearance, but as some of them have been seen to have three chief processes (Fig. 47), it is probable that all have. The axon, coming from the tapering end of the cell, is usually directed ventrad, and passes in this direction into the ventricle, as before described. In larvae 3 cm. long the structures of the mesencephalic roof have attained adult conditions.

At the posterior end of the body the spinal cord bends dorsad, and is continued into the heterocercal tail. The walls of the cord are here thin; in larvae of 2 to 3 cm. they are made up for the most part of a single layer of cells (Plate **8**, Fig. 61). In that portion of the central canal beyond the flexure there is a large number of characteristic posterior canal-cells. Roughly estimated they may number from 20 to 30; they are usually of the elongated spindle-shape, and frequently are seen to be continued into a neurite at each end (Fig. 61, *a*). The neurite, or axon, which runs forward is the larger and is always present. These axons run parallel for a considerable distance before uniting (Fig. 61, *ax.*). The cells give off a number of dendritic processes, which pass for the most part to the ventral side of the canal and enter the cord (Plate **6**, Fig. 43; Plate **8**, Fig. 61, *β*).

Cyprinidae (37). In young Notropis cornutus (a shiner) 2 cm. long (Plate **7**, Fig. 48, *fas. Reis.* See also Sargent, **:03** *b*, Fig. 23), the optic reflex apparatus is fully established. In the adult of 6 cm. Reissner's fibre is relatively large (diameter 1.5 micra), and well defined in its course through the canal and brain ventricles. The fibre emerges almost as a whole from just in front of the posterior commissure, but is joined by some fine fibrils coming from the pocket just behind and above the commissure.

There are no conspicuous cells in the anterior portion of the roof of the mesencephalon, and the constituent axons of Reissner's fibre have not been traced directly to the cells of origin. It is probable, however, that the cells of the apparatus lie wholly within the torus longitudinalis. Small fascicles of fibres make their way from the more anterior and ventral portions of the torus lobes into the recess between the lobes of the torus and above the posterior commissure (Fig. 48). A considerable tract of fibres comes from the anterior and deeper portions of each lateral lobe of the torus, and bending ventrad, the two meet in the median plane, and pass toward and into the ventricle anterior to the posterior commissure (Fig. 48, *trt. tor. fbr. Reis. a.*). The bundle becomes more and more compact as it nears the ventricle, and enters it as an apparent single fibre, the chief constituent of Reissner's fibre. Though the evidence is not conclusive, it seems probable that Reissner's fibre also receives elements from the ganglia habenulae, for fine filaments, which probably unite with Reissner's fibre, have been found in the ventricle just below the pars intercalatus (Fig. 48, *prs. i'cal.*).

In adult Abramis crysoleucus (roach), Reissner's fibre has a diameter of 3.5 micra. It is easily traced through the canal and ventricles. In the anterior part of the mesocoele Reissner's fibre is split into a main trunk and a number of finer branches. The latter emerge from the roof of the ventricle, some anterior and some posterior to the posterior commissure, and converging unite with the main trunk. The main trunk emerges into the ventricle a short distance anterior to the posterior commissure.

Within the brain tissue the main trunk of the fibre immediately breaks up into fine divisions, which can be followed but a short distance because of numerous neuroglia fibres which lie in their paths and obscure the course of the individual fibres. A group of rather conspicuous cells lies at the anterior end of the roof of the mesencephalon, *i. e.* the torus, lateral to the median plane. Numerous fibres from this group of cells run toward the ventricle where Reissner's fibre enters the brain. As no direct connection has been seen, it is doubtful whether these cells give rise to Reissner's fibre.

Cyprinus niloticus. In specimens 8 cm. long Reissner's fibre has been traced through its course to where it enters the brain.

SALMONIDAE (64). In the Salmonidae I have studied the optic reflex apparatus in three species, — the salmon (Salmo salar), the brook trout (Salvelinus fontinalis), and the lake trout (Cristivomer namaycush). Between the first two there are no essential differences, but the last shows minor variations. In all, the apparatus is developed early. It is far advanced at the time of hatching, and becomes fully established and functional during the first day of larval life. As might be expected, the young fry are active and respond readily to optical stimuli.

The morphology of the anterior portion of the mesencephalic roof in this family calls for some description. The tectum opticum attains a relatively enormous development both in area and thickness (Fig. *F*). This is probably to be directly correlated with the activities of these animals, and the consequent high value placed upon them as game fishes. As the result of its increased development, relative to other parts of the brain, the part which is cephalad and dorsad to the posterior commissure bulges forward (Plate **7**, Fig. 51. See also Sargent, :**03** ^{b}, Fig. 20). The mesocoele is prolonged into an elongated conical recess extending dorsad of the commissure (*rec. ms'coel.*). The tectum has so great a thickness that it extends below the torus longitudinalis (Plate **8**, Fig. 60), and in Salmo and Salvelinus meets the mesal edge of the tectum of the opposite side, so that the torus is enclosed between them (Figs. *E*, *F*). The two mesal edges of the tectum are more closely applied in their anterior por-

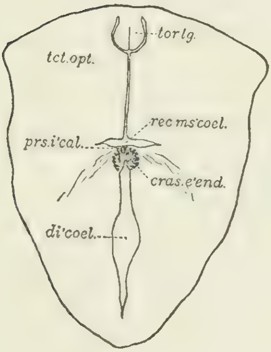

Fig. *E*.

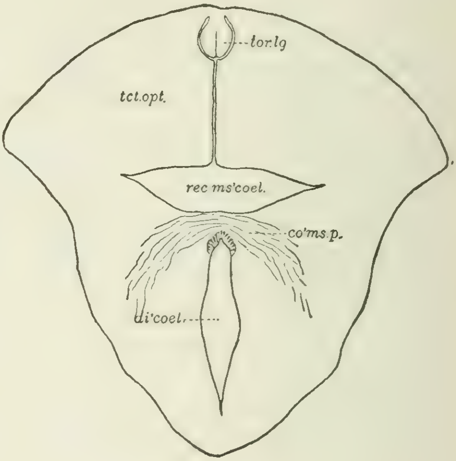

Fig. *F*.

Figure *E*. Salvelinus fontinalis, larva, 25 mm. long. Transverse section through the anterior extremity of the mesencephalon, showing the torus longitudinalis enclosed between the halves of the tectum.

Figure *F*. Salvelinus fontinalis, larva, 25 mm. long. Transverse section, caudad of the preceding, through the posterior commissure. For meaning of abbreviations, see Explanation of Plates, p. 257.

tions than elsewhere. As a result of the lateral pressure, the torus has become flattened laterally (Fig. 60), so as to appear in transverse sections dorso-ventrally elongated, and the median longitudinal fissure, dividing the torus into two lateral lobes, has almost disappeared.

Auerbach (**'88**, p. 376) failed to recognize the torus in the Salmonidae. In describing the sixty-day trout larva, he says, "Der Torus longitudinalis fehlt." In his figure, however, he has obviously shown the torus, describing it as "der in das Tectum eingebettete Zellstrang." That which he designates as the "Anlage des Torus longitudinalis" is the inner and ventral edge of the tectum, where it meets the mesal edge of the tectum of the opposite side below the torus (Figs. *E*, *F*).

The cells of the optic reflex apparatus lie wholly within the torus longitudinalis, of which they constitute the great mass (Fig. 60). They are spindle-shaped and apparently bipolar, their long axis extending vertically. The nuclei of the cells are relatively large, making up the greater mass of the cell body. The cytoplasm is small in amount, stains lightly, and is not sharply outlined, except at the two opposite poles of the cell, where the neurites pass off.

The neurites from the dorsal ends of the cells are fine delicate fibres without medullary sheaths. Proceeding dorsad, these fibres become aggregated into fascicles, forming two fibre-tracts, one on either side of the median plane, the tractus toro-tectalis. At the upper level of the torus they turn laterad and enter the tectum on either side (Fig. 60, *trt. tor. tct.*). Where they leave the torus the bundles are compact, but within the tectum they become more diffuse and break up in the superficial fibre-zone of the tectum. These tracts were first seen in teleosts by C. L. Herrick (**'91**). Their appearance led him to designate them as "the gelatinous tracts of the torus," and to assign to them a neurogliar nature.

The ventral neurites, the true axons, enter the mesocoele to form Reissner's fibre in two ways. The axons of those cells which lie in the ventral and posterior parts of the torus, near the median plane, enter the ventricle from the median ventral fissure of the torus, — tractus toro-fibrae Reissneris *posterior* (Plate **7**, Fig. 51; Plate **8**, Fig. 60, *fas. Reis.*). In Cristivomer, where the tectum is less developed and the mesencephalic roof less compact, most of the constituent axons of Reissner's fibre enter the ventricle from the median fissure. These axons in the larvae are not aggregated into bundles, but make their way singly into the mesocoele, where they coalesce to form larger trunks, all of which eventually unite to form Reissner's fibre. In Salmo and Salvelinus the portion of the mesocoele where the axons enter is reduced to a mere canal, owing to the union of the halves of the tectum below the torus (Figs. *E*, *F*). Through this the finer trunks of Reissner's fibre make their way into the anterior recess of the mesocoele.

The cells lying in the anterior, dorsal, and lateral portions of the torus send their axons into the ventricle by the shortest path. In the growth of the tectum cephalad these cells have come to lie far cephalad of the posterior commissure, so that the shortest path to the ventricle lies anterior to the posterior commissure (Plate **7**, Fig. 51; Fig. *K*). The axons taking this course are aggregated into two lateral bundles, the tractus toro-fibrae Reissneris *anterior*. These tracts, converging toward the median plane, enter the ventricle as compact bundles, in which the constituent axons can be recognized only in very early stages of development. In larval stages this tract within the ventricle consists of a number of divisions, which in the adult become consolidated, so that the greater portion of Reissner's fibre emerges into the ventricle at one point (Fig. 51). In Salmo and Salvelinus the greater number of the constituent axons of Reissner's fibre enter the ventricle by this path (*trt. tor. fbr. Reis. a.*).

Ventral and caudad to the posterior commissure these two sets of constituents meet and coalesce. In larval stages the anterior portion of Reissner's fibre consists of many divisions, and one frequently finds in sections lying within the mesocoele loosely tangled masses of these fibre-like divisions which have been displaced in cutting. This diffuse branching of Reissner's fibre is much reduced in older fry, and in the adult only a few small divisions may be found before the main trunk of the fibre enters the brain tissue anterior to the posterior commissure.

In the adult, Reissner's fibre in its course through the mesocoele is surrounded by a loose membranous sheath, which extends almost to the metacoele. This is formed from the membrane lining the ventricle by its deflection outward upon the fibre.

In its course through the brain ventricles and central canal Reissner's fibre appears as a sharply defined rod of conspicuous size. In trout fry 2 cm. long, the rod has a diameter of 0.3 micra. In the adult trout the diameter is 3 micra.

There is no well-marked ventriculus terminalis in the Salmonidae but at the posterior end of the canalis centralis, where the spinal cord is bent upward into the heterocercal tail, the posterior canal-cells can with some difficulty be distinguished, attached by their axons to the fibre as grapes to their stem.

Poeciliidae (92). In adult Fundulus heteroclitus Reissner's fibre is 1.0 micron in diameter, and has been followed through its whole course. The torus longitudinalis is well developed, especially in the anterior part of the mesencephalon, and hangs suspended freely in the mesocoele below the level of the tectum.

In larvae 1 cm. long, the median roof anterior to the posterior commissure (the 'Schaltstück' of Burckhardt) remains in a state of primitive simplicity (Plate 8, Fig. 59, *prs. i'cal.*). As in many other species, the cells of the torus are arranged in radiating columns, separated by ependymal fibres (*tor. lg.*). The fibre-like bundles of axons (*trt. tor. fbr. Reis. a.*) coming from the anterior part of the torus pass between its spindle-shaped ependymal cells (*cras. e'end.*) to enter the ventricle, where they unite to form the greater portion of Reissner's fibre (Fig. 59, *fbr. Reis.*).

There is no marked ventriculus terminalis, but in the slightly dilated posterior extremity of the canalis centralis there is a chain of about a dozen small posterior canal-cells.

Amblyopsidae (93). This group includes a number of species of small cave-inhabiting fishes having degenerate eyes; some of them are totally blind (see Eigenmann, '99, :03). The four species examined[1] afford a series in which the effect of disuse of the visual organs is shown by profound changes in the central nervous system (compare Figs. *G* and *H*), accompanied by the degeneration of the optic reflex apparatus.

Chologaster papilliferus has functional eyes, greatly reduced in size, the maximum diameter being 0.8 mm. The optic lobes are relatively much smaller than in the closely related Fundulus, which has normal eyes.

The tectum is thin and the torus-lobes but little developed. Reissner's fibre has in the adult a diameter of approximately 0.6 micron, being relatively much smaller than in the related Fundulus.

The three other species are totally blind. In Amblyopsis the vestigial eye has a maximum diameter of 200 micra; in Typhlichthys, 180 micra; and in Troglichthys, 85 micra (Eigenmann, '99). In the two latter species I have been unable to distinguish the slightest trace of Reissner's fibre. The optic centres of the central nervous system of Amblyopsis have recently been studied by Ramsey (:01), who finds the optic lobes and tracts distinctly degenerate; the tectum is only one-half to one-third as thick as in the normal brain. The torus longitudinalis is in a state of arrested development, similar to that of other teleosts before hatching, and scarcely exists as an independent structure (Plate 9, Fig. 64). It is marked off from the tectum by a shallow fissure, and contains few or no nerve-cells, consisting almost wholly of ependyma. The dorsal decussation of the tectum passing through it occupies the greater part of its thickness (Fig. *G*, *dec. d.*).

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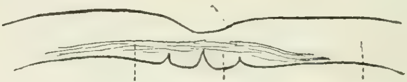
[1] I am greatly indebted to Prof. C. H. Eigenmann for the privilege of examining his extensive collection of preparations of blind fishes. He also kindly provided me with fresh material of Chologaster and Amblyopsis.

Reissner's fibre has been found only within the central canal, where it is an exceedingly delicate filament, which, in young specimens 8 to 10 mm. long, has a diameter of 0.1 micron (measured with a Zeiss micrometer ocular and apochromatic objective). In larger specimens, 30 mm. long, the diameter was somewhat greater. The constituents of the fibre in this case probably come in large part if not wholly from the ganglia habenulae, though I have been unable to trace a direct connection in this species. As the eyes in Amblyopsis are totally useless, the much-reduced Reissner's fibre is probably, as in the blind Myxine, exclusively a reflex fibre-tract between the olfactory centres of the habenulae and the musculature. With this degeneration of the optic reflex apparatus there is apparently an increased development of the reflex apparatus of which Mauthner's fibres are the conducting tracts.

Fig. *G.*



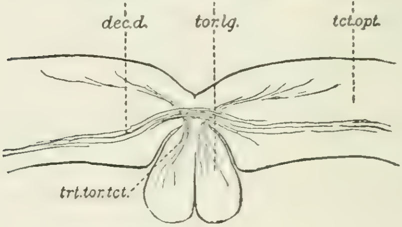


Fig. *H.*

Figure *G.* Amblyopsis spelaeus, adult. Transverse section of the tectum opticum and torus longitudinalis.

Figure *H.* Fundulus heteroclitus, adult. Transverse section of the tectum and torus. For meaning of abbreviations, see Explanation of Plates, p. 257.

In all these blind species Mauthner's fibres are abnormally large. This naturally comes about with the greater importance given the auditory sense accompanying the degeneration of the visual sense.

Esocidae (94). In Tylosurus marinus the torus longitudinalis extends through the whole length of the elongated optic lobes. Several branches of Reissner's fibre emerge from the 'Schaltstück,' just anterior to the posterior commissure. Another fine branch has been traced still farther forward nearly to the base of the ganglia habenulae. In specimens 20 cm. long, Reissner's fibre has a diameter of 2.5 micra.

Gasterosteidae (98). The mesencephalon of Gasterosteus bispinosus is short and very compact. The tectum is so thick and the torus so largely developed that the mesocoele is greatly reduced. In the adult the torus is crowded down and partly around the posterior commissure (Plate **10**, Fig. 69). The cells of the torus are small and closely crowded. At the anterior end a bundle of fibres passes out of each torus lobe into the canal-like fissure between the torus and the posterior commissure. The greater number of axons from the torus converge into close bundles in the anterior portion of the torus lobes (*trt. tor. fbr. Reis. a.*) and, traversing the 'Schaltstück,' enter the ventricle in the median plane anterior to the posterior commissure. These different trunks converge and fuse beneath the posterior commissure, and farther caudad are joined by the posterior trunk. There is some evidence that a trunk made up of axons from the ganglia habenulae also joins Reissner's fibre (Fig. 69). The consolidated fibre (*fbr. Reis.*) has in the ventricles and anterior part of the canal a diameter of 1.2 micra (see also Sargent, :**03**^{b}, Fig. 22).

Atherinidae (106). The optic reflex apparatus has been studied in many individuals of the genus Menidia (species gracilis and notata). Sections of a number of stages from 1 cm. to 10 cm. in length have been cut in the three principal planes. In the youngest specimens examined the apparatus is already developed, and Reissner's fibre, though very small, is sharply defined.

The torus longitudinalis (Plate **8**, Fig. 58) is of large size, its dorso-ventral thickness being as great as that of the tectum, below which it projects into the mesocoele, largely filling that space. As the torus hangs suspended freely in the mesocoele, there is no lateral pressure on it, as in the Salmonidae, and its flattening is consequently dorso-ventral. The cells of the optic reflex apparatus make up the mass of the torus, and are oval or nearly spherical in form, with their long axes lying in the direction of the outgoing axon. In the young specimens the axons of the cells near the ventral surface of the torus pass directly into the mesocoele along the whole ventral surface of the torus, but more numerously near the median plane. In the ventricle these coalesce and run together in the median plane forming a diffuse dendritic system of trunks and branches (Fig. 58, *fas. Reis.*). In older specimens the number of branches is greatly diminished. The cells from the dorsal and anterior parts of the torus send their axons into the ventricle cephalad of the posterior commissure. At an early stage of development these axons emerge from the whole upper surface of the ventricle singly or in numerous small trunks, which are directed caudad and toward the median plane. These unite into a single trunk which, caudad of the posterior commissure, unites with the other system to form Reissner's fibre. In the adult the whole mass of axons in front of the posterior commissure emerges into the ventricle as a few consolidated trunks.

At its posterior termination the canalis centralis expands into an ovoid, almost spherical, ventriculus terminalis. In the canal beyond the heterocercal flexure I have found a number of small posterior canal-cells.

In the nearly related families examined no important observations have been made. In the species Siphostoma fuscum, Mugil cephalus, Ammodytes americanus, Scomber scombrus, and Sarda sarda, Reissner's fibre is of small size, but has been found and traced for greater or less distances through the canal and brain ventricles.

In the rudder fish, Naucrates ductor, Reissner's fibre has a diameter of 2.5 micra.

Pomatomidae (126). Reissner's fibre in the bluefish, Pomatomus saltatrix, is of large size, having in the adult a diameter of 8 to 9 micra in its course through the anterior portion of the canal and brain ventricles. In the mesocoele below the valvula cerebelli, it is divided into two or three main trunks (*fbr. Reis.* Fig. *I*). One passes above the posterior commissure (*trt. tor. fbr. Reis. p.*), and between it and the valvula cerebelli into the fissure between the lobes of the torus, and there again divides into two lateral branches, which enter the lobes of the torus, where they break up into fibre-tracts which have their origin in the cells of the torus. The other main trunk continues cephalad, ventral to the posterior commissure and below the 'Schaltstück,' and divides several times (*trt. tor. fbr. Reis. a.*). The divisions enter between the ependymal cells and thence run dorsad and laterad into the torus longitudinalis, where they break up into their constituent axons. One or two fine trunks have been traced cephalad nearly to the base of the ganglia habenulae.

Stromateidae (135). In the young butterfish, Rhombus triacanthus, 15 mm. long, the torus longitudinalis is well developed anteriorly (Plate **7**, Fig. 50). The growth of the valvula cerebelli forward into the mesocoele has resulted in the crowding forward of the torus so that the latter lies almost wholly dorsal to the posterior commissure, and within the anterior prolongation of the mesocoele above it. Reissner's fibre is formed very largely from trunks emerging from the 'Schaltstück' anterior to the commissure (*trt. tor. fbr. Reis. a.*). In addition there are clearly some finer trunks which emerge from the anterior extremity of the 'Schaltstück,' and probably have their origin in the ganglia habenulae. In the mesocoele below the valvula, Reissner's fibre is fully consolidated and 0.7 micron in diameter. In the closely related Eupomotis gibbosus, the conditions are practically the same as in Rhombus. In the adult the diameter of the fibre is 3 micra.

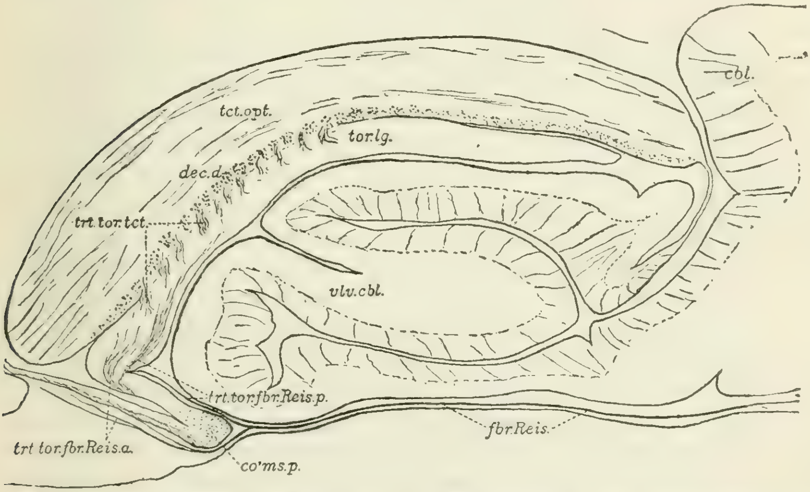


Figure *I*. Pomatomus saltatrix, adult. Sagittal section of the dorsal portion of the midbrain. For meaning of abbreviations, see Explanation of Plates, p. 257.

Serranidae (146). Of this family I have studied the apparatus in the adult of the white perch, Morone americana (Plate **10**, Fig. 68), and the sea bass, Centropistes striatus. In both, the importance of the apparatus is evidenced by the size of Reissner's fibre. In the perch its diameter is 3 micra, in the bass 4 micra. The mesocoele is occupied by the large valvula, so that the torus extends only a short distance posterior of the commissure. The axons from the torus-cells pass in many small fascicles toward the ventricle by the shortest path through the 'Schaltstück' and emerge obliquely into the ventricle in front of the posterior commissure in many small trunks which immediately unite to form Reissner's fibre. Other small trunks (Fig. 68, *fbr. hab. Reis.*), emerging from the anterior portion of the 'Schaltstück,' doubtless come from the habenulae.

Sparidae (151). In this family the conditions are very similar to those in the preceding groups, but a few points of difference may be mentioned. In the scup, Stenotomus chrysops, the ependymal cells of the 'Schaltstück' are continued in long, coarse fibres, which run from the ventricle obliquely toward the torus and tectum. This makes it difficult to distinguish and follow the constituents of Reissner's fibre. It has, however, been determined that numerous fine trunks from the torus-lobes converge toward the median plane and, passing obliquely caudad and ventrad, fuse and emerge as the consolidated Reissner's fibre at a point immediately below the posterior commissure. The membrane lining the ventricle in this region is continued out over the fibre as a thin, loose, membranous sheath, which extends halfway through the mesocoele. The relatively large fibre is 3 micra in diameter.

Sciaenidae (155). In the squeteague, Cynoscion regalis, the optic lobes are of great size, and extend far in advance of the posterior commissure, resulting in a much more capacious mesocoele than in the preceding forms. The torus-lobes hang suspended freely in the spacious pocket of the mesocoele above the posterior commissure (Plate **9**, Fig. 63, *tor. lg.*). At their anterior margin the lobes fuse with the posterior commissure. Caudad of the commissure and above the valvula, the lobes rapidly diminish in size (see also Sargent, **:03**^{b}, Figs. 15–19).

As the great mass of the torus lies above the posterior commissure, the axons of its cells find their way to the ventricle almost exclusively through the 'Schaltstück.' It is doubtful, in fact, if any remnant of the pre-commissural tract remains. The axons of the torus-cells run forward through the torus-lobes in small fascicles, which at the anterior end of the torus are consolidated into trunks in which the separate axons can no longer be distinguished. These bend around the posterior commissure converging in the median plane with those of the opposite side. They traverse the 'Schaltstück' obliquely, ventrad and caudad, passing between the elongated ependymal cells, and undergoing fusion, so that before entering the ventricle they are consolidated into three definite trunks. The more posterior of these is much the largest. In one specimen these trunks, beginning with the most posterior, measured, 5, 4, and 2 micra respectively in diameter. These trunks before emerging into the ventricle run for some distance caudad just under the membrane limiting the ventricle, and emerge successively just under the posterior commissure. Within the mesocoele they are joined by some finer branches from farther forward, with which they coalesce to form Reissner's fibre. This has a diameter of 8 micra. The membrana limitans interna, formed by the ependyma which covers the 'Schaltstück,' is deflected outward over the fibre, forming a loose, wrinkled, membranous sheath, extending nearly to the cerebellum.

Reissner's fibre is of such size in this species that it has been found practicable to follow its course through the ventricles and canal in series of transverse sections, though it often requires careful search to distinguish it among the coagulum and blood corpuscles about it. In transverse sections the portions of the fibre appear as cylinders, viewed from the end. These are often toppled over, or otherwise displaced, or they are sometimes missing, having dropped out in the process of making the preparation.

In one series of sagittal sections studied, the fibre was found irregularly coiled within the fourth ventricle, and the adjacent anterior portion of the central canal contained no fibre. The coiled portion of the fibre was shrunken and thicker than the normal fibre where the course was straight. The coiled portion was from 12 to 14 micra in diameter, the normal fibre 9 to 10 micra. In this case the roof of the ventricle was entire, so the fibre could not have been drawn out of its natural position by any external force. When in the fresh condition the spinal cord was severed 2 cm. posterior to the brain, the fibre probably by its own elasticity recoiled into the ventricle. This shrinking and coiling was doubtless accentuated by the action of Flemming's fluid, in which the preparation was fixed.

The large size of Reissner's fibre in this species makes it a favorable subject for the study of its finer structure. In thin transverse sections it is seen to have a very definite internal structure. There is a thin medullary sheath about the fibre, having a thickness one-tenth to one-twelfth of the diameter of the fibre (Plate 7, Fig. 53). This takes stains in precisely the same manner as the myelin of ordinary medullated fibres. The central portion of the fibre has in section a finely punctate appearance, suggestive of its fibrillar nature (Fig. 54). Between this central portion and the medullary sheath there is a clear hyaline ring. In sections double stained with Congo red and Ehrlich's hematoxylin, the sheath takes the blue deeply, the central portion the red, the hyaline ring remaining unstained. The medullary sheath is continued over the divisions of the fibre to where they enter the 'Schaltstück' and divide.

Within the central canal numerous fine fibrils are given off from the fibre to the walls of the canal. It is very difficult at times to distinguish the fine filmy artifacts formed about the fibre from the actual processes.

Labridae (160). An extensive collection of preparations of Tautogolabrus adspersus has been drawn upon for the study of the optic reflex apparatus. Unfortunately, however, this is not a very favorable species for this investigation, as the brain reaches a high degree of teleostean complexity. The optic lobes are large and the tectum of great thickness. The torus, though relatively not so large as in the preceding forms, is well developed. The lobes of the torus remain quite distinct (Fig. *J*) instead of fusing, as in the Salmonidae and other forms. Anteriorly, where they are suspended in the mesocoele below the level of the tectum, they have in section a rounded outline (Fig. *J*, *tor. lg.*). Posteriorly they taper away and lie more completely between the halves of the tectum. Above the posterior commissure they are closely applied to it and fuse with it (Plate **8**, Fig. 57. See also Sargent, **:03**^{b}, Figs. 6, 7).

All portions of the apparatus are of small size, Reissner's fibre being always less than 1 micron in diameter and consequently difficult to find. The difficulty of studying the anterior ending of this apparatus is complicated by the presence of blood vessels in the ventricles of the optic lobes. Large blood vessels run from the floor obliquely upward through the lateral horns of the optic ventricle and branching enter the tectum.

In young specimens 2 to 3 cm. long, Reissner's fibre may be found through the entire length of the central canal to the extreme end. The apparatus is probably fully developed at the time of hatching or shortly after. The cells of the torus are small, of uniform size, oval or spherical in form, and have in general a bipolar appearance. They are for the most part arranged in radiating bands or columns separated by neuroglia fibres (Plate **8**, Fig. 55, *tor. lg.*).

The axons of the cells find their way into the ventricle by one of two paths, — one entering the ventricle anterior to the posterior commissure, the other entering dorsal and posterior to it (Fig. *K*). In the larval tautog, 2 to 3 cm. long, the axons from the cells lying near the median ventral surface of the torus enter the ventricle more or less directly along the median ventral groove of the torus, but mostly at the anterior end. This is shown in Figure 55, where numerous fine axons (*fas. Reis.*) from the cells of the torus are seen entering the ventricle in the median groove of the torus-lobes. As development proceeds, the separate axons become aggregated into bundles. In the adult the axons enter the groove as five or six bundles (Fig. *K*, *trt. tor. fbr. Reis. p.*), which, running ventrad and caudad, unite with a similar bundle from the opposite side.

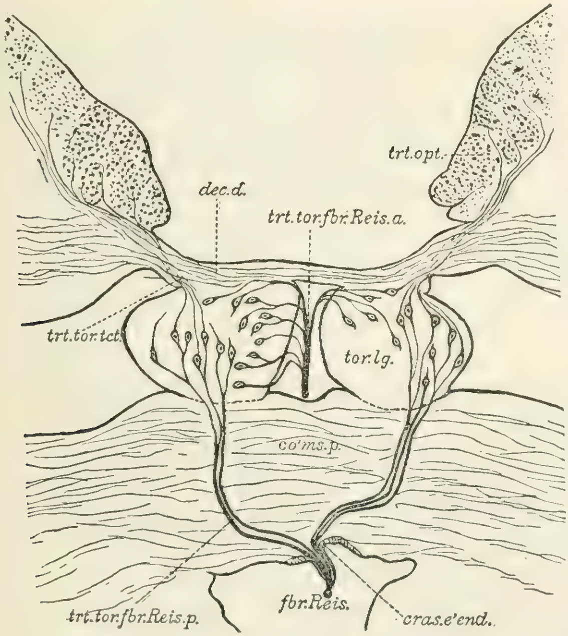


FIGURE *J*. Tautogolabrus adspersus, adult. Diagrammatic transverse section of the anterior median dorsal portion of the mesencephalon and torus longitudinalis, through the posterior commissure. The end apparatus of Reissner's fibre is projected on a plane. (In this figure, for *trt. tor. fbr. Reis. a.*, read *trt. tor. fbr. Reis. p.*, and *vice versa.*) For meaning of abbreviations, see Explanation of Plates, p. 257.

The cells from the more lateral and anterior portion of the torus send their axons into the ventricle through the posterior commissure or just anterior to it. In young larvae before the development of the commissure this is the shortest path to the ventricle (Fig. 56, *trt. tor. fbr. Reis. a.*), and the axons penetrate to the ventricle as a rule singly. As the commissure develops in thickness, this becomes a longer path, and the axons become aggregated into fascicles and bundles. Later in front of the tract this posterior commissure becomes much more closely consolidated. The tracts from each half of the torus are shown in Figures *J* and *K*, passing through the anterior part of the torus and converging to the median plane, where they emerge into the ventricle in one or two separate divisions just anterior to the posterior commissure. I have been successful in getting this tract impregnated in Golgi preparations, though from a large collection of Golgi material I have gotten no impregnation of other portions of the torus longitudinalis.

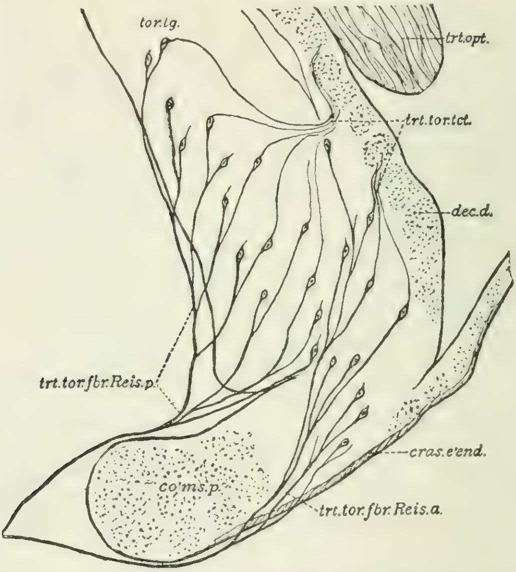


FIGURE *K*. Tautogolabrus adspersus, adult. Diagrammatic parasagittal section of the anterior, median, dorsal portion of the mesencephalon. The end apparatus of Reissner's fibre of one side is projected on the median plane. For meaning of abbreviations, see Explanation of Plates, p. 257.

Processes of another kind arise, usually from the dorsal sides of the torus cells. These run dorsad and toward the median plane and collect in fascicles, forming the tractus toro-tectalis (Figs. *J*, *K*; Plate 8, Fig. 57, *trt. tor. tct.*), which at the upper level of the torus turn outward into the tectum. Some of the fibres mingle with those of the dorsal decussation (*dec. d.*), while others are lost in the tractus opticus (*trt. opt.*) of the tectum. It is through these tracts that the apparatus is put in direct connection with the endings of the optic nerve-fibres.

In Tautoga onitis, a much larger species than the preceding, but closely related to it, the apparatus is without essential differences. Reissner's fibre within the canal is from 4 to 5 micra in diameter, the lumen of the canal being 25 micra in diameter.

Balistidae (169). In young Balistes vetula, 2 cm. long, the torus is short and not well developed. The ependymal cells of the torus are prominent, and the nerve-cells relatively few. The axons of the median ventral torus-cells pass into the fissure between the torus-lobes in numerous fine trunks, and behind the posterior commissure unite with the trunks which enter the ventricle through the 'Schaltstück.' Reissner's fibre is a delicate thread 1 micron in diameter.

Monacanthidae (170). In a young specimen of Alutera schoepfii 12 cm. long the torus was somewhat better developed than in Balistes. Six to eight delicate fibre-like trunks emerge from the 'Schaltstück' and running parallel gradually unite (Plate **7**, Fig. 49). Even under the valvula, however, several parallel trunks were found (*fas. Reis.*). These unite beneath the cerebellum to form Reissner's fibre, whose diameter is from 1.5 to 2 micra.

Cottidae (179). Both young larvae and adults of the sculpin, Myoxocephalus aeneus, have been studied. In larva 15 mm. long Reissner's fibre in the rhomboidal fissure measures 1 micron in diameter. In the adult it has a diameter of 6 micra. It is formed from one chief trunk emerging from the 'Schaltstück' under the posterior commissure, and a number of small trunks which enter the mesocoele above the posterior commissure, in the fissure between the lobes of the torus. All these unite and fuse immediately behind the commissure.

Cyclopteridae (182). In the one adult Cyclopterus lumpus studied, the torus was but little developed. Numerous fine fibre-like trunks emerging into the ventricle in the median plane from the whole length of the 'Schaltstück' unite immediately to form Reissner's fibre, whose diameter is 3 micra. Some of these trunks come from so far forward that it is unlikely that they have their origin in the torus, but rather in the ganglia habenulae.

Triglidae (184). In the two specimens of the sea-robin, Prionotus strigatus, examined, Reissner's fibre measures, respectively, 2.5 and 3.5 micra in diameter. The fibre enters the ventricle as a whole just anterior to the posterior commissure.

Malacanthidae (190). In the tilefish, Lopholatilus chamaeleonticeps, the metencephalon and myelencephalon only were available for study. Reissner's fibre is of large size, measuring from 5 to 8 micra in diameter.

Batrachoididae (198). In the toadfish, Opsanus tau, Reissner's fibre has a diameter in the canal of 1.5 micra, the lumen of the canal being 45 to 50 micra in diameter.

Gadidae (214). Four species of this family have been studied, both in larval and adult stages. In the larvae of the common cod, Gadus callarias and Gaidropsarus argentatus, from 1 to 3 cm. long, Reissner's fibre has a diameter of 0.5 to 1.0 micron. It is formed from numerous fine fibre-like bundles of axons of the torus-cells, most of which enter the ventricle through the 'Schaltstück' (Plate **7**, Fig. 52), a lesser number emerging from the anterior end of the ventral fissure of the torus.

In Microgadus tomcod, at the anterior end of the mesocoele (Plate **10**, Fig. 70), Reissner's fibre breaks into divisions which enter the brain tissue separately. The main division enters immediately cephalad of the posterior commissure, breaking up into many fibrils as soon as it is within the tissue. These fibrils are made up largely of the axons of the torus-cells, which lie immediately anterior and dorsal to the posterior commissure. Another branch enters the brain tissue anterior and dorsal to the commissure, and breaks up into numerous sharply defined trunks, which, being intermingled with coarse neuroglia fibres (*fbr. e'end.*), are difficult to follow. One branch of the fibre was observed to pass directly through the commissure. This condition has been seen in other species, and at first seemed most anomalous. But as the cells of this apparatus have already sent their axons into the ventricle before the posterior commissure is fully developed, it is easy to see how, through the subsequent growth of the commissure, this condition may be brought about.

Pleuronectidae (219). The apparatus has been studied in sagittal and transverse sections of Paralichthys dentatus and Pseudopleuronectes americanus, in which there are no essential differences. The torus-lobes (Plate **9**, Fig. 62, *tor. lg.*) are distinct, flattened, and pad-like, having a somewhat rounded rectangular outline in cross-section. Posteriorly they are in close contact with the large valvula, and taper away at about the middle of the mesencephalon. (For the morphology of the torus see also Sargent, **:03**^{b}, Figs. 11–14.) The axons of the torus-cells run toward the dorsal and median line, fusing into close bundles, and some fine trunks pass into the median fissure above the posterior commissure. The rest of the trunks continue cephalad, and, curving ventrad, enter the ventricle on either side of the 'Schaltstück,' which is wide and thin. These, joining with other fine trunks from further forward, unite with those from the ventral fissure of the torus to form Reissner's fibre, whose diameter is about 3 micra. Through the posterior portion of the central canal Reissner's fibre gives off fine fibril branches. These run for the most part toward the ventral side of the canal, and have a course slightly oblique and caudad (Fig. 66, *a*). They enter the cord, passing between the large ependymal cells, and are there lost to view, the presence of numerous neuroglia fibres making it impossible to follow them.

LOPHIIDAE (221). Both young larvae and adult brains of Lophius piscatorius have been studied. In the 8 mm. larvae, one week after hatching, a delicate filament passing from the region of the posterior commissure through the ventricles was identified as Reissner's fibre. In the adult the spinal cord is short and the fibre tapers rapidly in its course through it. In the fourth ventricle the diameter of the fibre is 7 micra; in the central canal 5 cm., and farther, caudad the diameter is 3 micra.

D. CRITICAL DISCUSSION.

In the extensive literature of the teleost brain the mesencephalon has not been neglected. Its fibre-tracts and the structure of the tectum opticum have been minutely described by many investigators. However, the morphology of the anterior portion of the roof, including the torus longitudinalis, its finer anatomy, and its relations to surrounding structures, has received very inadequate treatment.

The torus longitudinalis was first described, in the herring, by Carus (**'14**), who believed it to be the homologue of the fornix of higher vertebrates, and so named it. Subsequently Gottsche (**'35**) agreed regarding this homology. Stieda (**'68**, p. 24) first applied the term torus longitudinalis. Baudelot (**'70**, p. 90) used the terms 'lame commissurale' and 'éminences commissurales' in referring to it. Fritsch (**'78**), who believed the optic lobes to represent the cerebral hemispheres, considered the torus longitudinalis to be homologous with the fornix and corpus callosum, and referred to it as the fornix.

Sanders (**'78**, p. 754), in giving the first description, in Mugil, of the finer structure of the torus, says, "The cells (Figure 19) which constitute the fornix are mostly of a spherical form, consisting almost entirely of nuclei with only a narrow rim of protoplasm around them. . . . Occasionally larger cells occur, which present a triangular shape from the greater quantity of protoplasm belonging to them. . . . These cells are arranged in rows, or in single files radiating from the upper and inner angle of the fornix; each row is separated from its neighbors by bundles of fibrillae, which also radiate from the same point; these bundles are thicker at the proximal end, and gradually become smaller by giving off radiating fibrils in their course. The cells are attached to these fibrils sometimes by short branchlets, and sometimes they are sessile."

Stieda (**'68**), Bellonci (**'80**, **'81**, **'82**), Mayser (**'82**), Wright (**'84**), Auerbach (**'88**), and C. L. Herrick (**'91**, **'92**, **'92**^{a}) treat of the optic lobes, but have little or nothing to say of the torus, and nothing whatever of its finer structure. Of the more recent writers treating of this region, the torus longitudinalis is not mentioned by Fusari (**'87**), Burckhardt (**'94**), Van Gehuchten (**'94**), Neumeyer (**'95**), Mirto (**'95**), Haller (**'98**), or Pedaschenko (**:01**), and receives mere mention from Ramsey (**:01**).

Rabl-Rückhard (**'82**, **'84**), from embryological and comparative anatomical studies, proved Fritsch to be in error, and showed the torus longitudinalis to be a structure peculiar to fishes. Later (**'87**), in studying the development of the torus in the salmon, he found it to be developed from the roof of the mesencephalon as a longitudinal thickening, by the multiplication of the cells of the inner layer of the tectum opticum. He believed the torus longitudinalis to be represented in the higher vertebrates by the thickened ependyma which he observed under the posterior commissure in amphibia, reptiles, and birds. In a later paper Rabl-Rückhard (**'94**) again emphasized this view. This erroneous interpretation has been accepted by Gage (**'93**) and Johnston (**:01**, **:03**), and has led to considerable confusion. The cells described by Johnston (**:01**, pp. 48, 145) as "Type C: cells of the torus longitudinalis Halleri," are more properly cells of the tectum opticum.

C. L. Herrick (**'91**, p. 172) says of the torus merely: "The structure of this body is very simple, consisting of dense clusters of small cells like Deiter's corpuscles." In common with previous observers he did not consider the torus a nervous centre. In a later paper (**'92**, p. 44), he says: "A considerable part of the fibres which enter the torus longitudinalis are not nerve-fibres, but arise from the modified epithelium of that body and pass to the ectal surface of the tectum. Such gelatinous tracts are to be regarded as mere persistent walls of the original single epithelium."

Sala (**'95**) studied the torus longitudinalis of the young of Tinca vulgaris by the method of Golgi, and demonstrated the presence of

(1) nerve-cells with characteristic nerve-processes, (2) a nerve-net distributed through the whole area of the torus, and (3) ependymal cells. The "cellules nerveuses speciales" (compare Fig. *L*) are globular or pear-shaped, 10 to 14 micra in diameter, with a large nucleus, and are irregularly disposed through the whole space of the torus. As a rule there is but a single process from the cell, which at a short distance from the cell divides. One of these processes is "plus delicat et plus tenu," and may again divide. "Il conserve un cours presque rectligne et . . . se laisse suivre sur le longues portions vers la parti supérieure et externe du torus, . . . sur le point où le torus s'attache lateralement

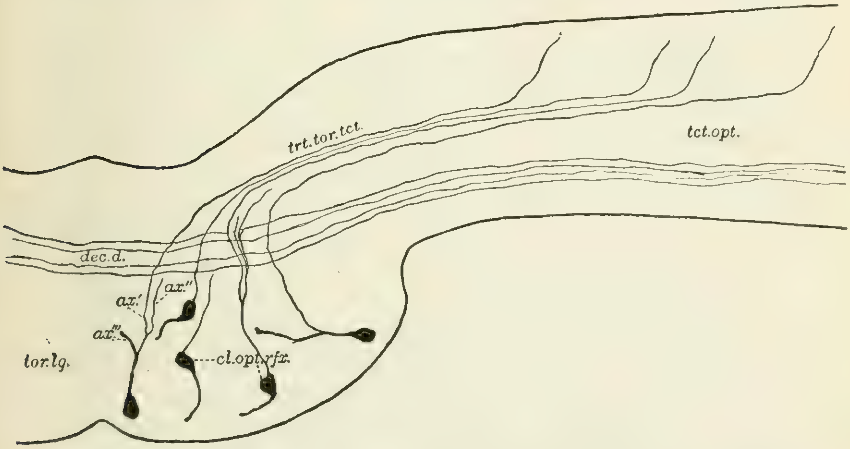


FIGURE *L*. Tinca vulgaris. Diagrammatic transverse section of the torus longitudinalis and adjacent portion of the tectum of the right side. (Adapted from Sala's Figs. 1, 2, and 3.) For meaning of abbreviations, see Explanation of Plates, p. 257.

avec le toit optique, . . . ou il contribué à constituer un faisceau de fibres nerveuses qui, se continue latéralement dans le toit optique." This description corresponds with the fibres which collectively I have called the tractus toro-tectalis (compare Fig. *L*, *trt. tor. tct.*).

According to Sala this tract in passing out of the torus into the tectum passes obliquely between the fibres of the dorsal decussation, "fibres commissurales des lobes optiques," and "plie brusquement à l'externe et se dirige vers le toit optique, courant toujours sur un plan un peu supérieur à celui sur lequel courent les fibres commissurales."

This fibre-tract from the torus to the tectum was first seen by

Bellonci ('**81**, p. 27, Tav. II. Fig. 1 (b)), who described it as follows: "Von dieser Schicht [the superficial layer of the tectum] gehen sehr feine blasse Faserbündel aus, welche, nachdem sie parallel der Oberfläche des Tectum verlaufen sind, dieselbe in schräger Richtung durchziehen, und sich theils in darunter befindlichen Region nach dem Tectum zu, theils im Torus longitudinalis allmählich auflösen, und zum Theil dazu beitragen, die obere Commissur des Tectum zu bilden."[1]

This tract was also seen by Fusari ('**87**, Taf. III. upper end of figure), who, however, did not trace its origin to the torus. It is the same as that which C. L. Herrick ('**92**, p. 43, Fig. 9) somewhat incorrectly described as the 'gelatinous tract,' and believed to be made up of neuroglia fibres. It is identical with the tractus toro-tectalis, which I have described in teleosts. Sala believed that the fibres of this tract passed out from the superficial layer of the tectum into the optic nerve. As Johnston (:**01**, p. 145) has pointed out, "the fact that they enter the superficial fibre-zone is not sufficient evidence that they go to the optic nerve." Recent investigations on the origin of the optic nerve-fibres (S. Ramon y Cajal, '**96**, p. 104, Fig. 46) show the impossibility of this.

Of the other branches which Sala found to come off from the torus-cells, one was similar to that already described, but could be followed only a short distance into the tectum (Fig. *L*, *ax.''*). This is evidently the incompletely impregnated neurite which goes to form the tractus tecto-cerebellaris. The third process, the greatest in diameter, he found to take an irregular course and to terminate freely a short distance from the cell in a slight enlargement (Fig. *L*, *ax.'''*). This, it is quite evident from its way of ending, and failure to establish relations with other elements, is due to its incomplete impregnation. Sala makes no attempt to explain the relations or functions of this process. I think there can be little doubt that this is the partly impregnated axon of the cell, which runs mesad and cephalad to emerge into the ventricle either in the ventral fissure of the torus, or anterior to the posterior commissure. A careful study of his figures shows that this process is usually directed toward the median plane (Fig. *L*). Unfortunately all his figures are of transverse sections, and evidently from the posterior part of the torus.

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[1] According to Bellonci the optic nerve-fibres end in the outer layer of the tectum, where they are lost in the fine net through which they are in direct connection with the long processes of the nerve-cells of the inner layer of the tectum. In another paper Bellonci ('**80**) gave numerous figures (Figs. 5, 11, 18, 28, 32) of cells from the optic tectum whose finer dendrites anastomose, in some cases (Fig. 18) anastomosing with the ends of the optic nerve-fibres.

In such sections it would be very difficult to determine the true course and relations of these axons, even if they were completely impregnated.

P. Cajal ('99) and Catois (:01) follow Sala closely in their interpretation of the torus longitudinalis. Catois (p. 130) describes, in addition, certain nerve-fibres which terminate in the torus, and, as he believes, are derived in part from the dorsal decussation and in part from the third layer of the tectum. These fibres end in connection with the dendrites of the torus-cells. Johnston in reviewing the literature of this subject says (:03, p. 1076): "Dass der Torus longitudinalis eine besondere Bedeutung habe, ist noch nicht endgültig gezeigt worden. Sala und Catois geben ihn als die Ursprungsstelle der centrifugalen Fasern in den optischen Nerven an, aber wie Referent nachgeweisen hat, sind diese Fasern bei Wirbeltieren vorhanden, bei denen der Torus fehlt. Seine gegenwart wird wahrscheinlich durch den Einfluss des oben beschrieben nucleus magnocellularis tecti veranlasst."

E. Summary for Teleosts.

The midbrain roof of teleosts is more complex in its structure than in the groups previously described. Projecting downward from the median roof of the mesencephalon is the torus longitudinalis, a paired ridge extending along the median roof of the optic lobes, and ontogenetically derived from the mesal edges of the tectum. Phylogenetically the torus has its beginning in the ganoids, the increase in size of the optic reflex cells and the pressure of other surrounding portions of the brain resulting in a down-swelling at the anterior end of the tectum forming two incipient lobes or ridges on either side of the median plane. In the primitive Siluridae and degenerate Amblyopsidae there is only a slight advance upon this condition. In the Salmonidae the torus lobes are laterally compressed and the median fissure obliterated by the pressure between the halves of the thick tectum. In nearly all other teleosts the torus-lobes hang suspended freely and more or less separately within the ventricle, assuming a considerable variety of forms in the different groups.

The cells of the optic reflex apparatus, in common with the other cellular elements of the brain, are of relatively small size in the teleosts, and are concentrated in the torus longitudinalis. Unipolar or bipolar in their general outline, they ultimately give rise to three processes forming three fibre-tracts on each side of the brain. The fascicles of fibres of the tractus toro-tectalis and tractus toro-cerebellaris running dorsad and cephalad pass out of the torus together into the tectum. The former tract crosses through the dorsal decussation and continuing laterally in the tectum breaks up in its ectal portion among the endings of the retinal fibres of the optic nerve. The tractus toro-cerebellaris turns laterally below the dorsal decussation and eventually takes an oblique course around the lateral border of the tectum to the cerebellum.

The third set of processes, the axons, have a generally anterior and ventral direction, eventually entering the ventricle and by fusion forming the fibre of Reissner. In the more primitive teleosts the nerve-fibres enter the ventricle in the anterior portion of the median fissure between the torus-lobes. In embryonic and early stages the axons enter the ventricle more or less singly, but as development advances they become consolidated into a smaller and smaller number of fascicles, in which the constituent axons are no longer distinguishable.

In the more highly differentiated teleosts the mode of formation of Reissner's fibre is more complex. The anterior portion of the optic lobes and torus project cephalad of the posterior commissure and overlie the diencephalon. The torus-cells of this region send their axons, as the tractus toro-fibrae Reissneris *anterior*, by the shortest path into the ventricle, through the ependymal thickening of the pars intercalatus. Axons of cells in other portions of the torus take the more primitive path to the ventricle and constitute the tractus toro-fibrae Reissneris *posterior*, which enters the ventricle in the narrow recess between the lobes of the torus posterior (dorsad) to the posterior commissure.

Some fine branches coming from the base of the ganglia habenulae run caudad through the diencephalon in the ependymal groove below the 'Schaltstück.' Caudad of the posterior commissure all these trunks unite to form the fibre of Reissner, which runs posteriorly through the central canal, and gives off branches to the cord through the posterior two-thirds of its course. At its posterior extremity it is in connection with a system of posterior canal-cells, which are smaller but more numerous than in selachians.

II. Physiological.

A. Comparative Physiology.

1. *The 'Flight Reflex.'*

The physiological action of this optic reflex apparatus has been observed for many years, though nothing was known of the apparatus by which these reactions were brought about. Edinger ('**99**, p. 279) says: "The conduct of the youngest brood of fish, still attached to the yolk-sac, as they swim about is doubtless regulated by law with regard to light. Most probably the phenomenon usually called 'flight' should be classed among 'tropisms.' It is present at a time at which a developed nervous system is out of the question." Again, p. 285, "A peculiarity present in even the youngest brood of fish is a recoil from sudden optical or other light impressions. This 'flight reflex,' as we shall call it for the sake of brevity, is retained by all fishes beyond the stage of maturity."

This flight reflex is a striking and interesting phenomenon in many fishes. I have studied it experimentally in many species of young fishes in the aquaria of the U. S. Fish Commission at Wood's Hole, but one illustration will suffice. A tank 6 x 4 x 4 feet contained some 50 or 60 young mackerel, 3 to 4 inches long, which had recently been taken. They responded instantly and almost as a unit to the slightest movement on the part of a person standing before the tank. If the extended arm were moved horizontally to the right, the school of fish instantly and in unison moved horizontally to the left. If the movement of the arm were downward, the fish moved upward, or if an oblique movement were made, the fish responded promptly by a movement obliquely in the opposite direction. As the hand was withdrawn, the fish followed it back, as if actuated by some force of attraction, but probably because, the stimulus for flight being removed, they tend to disseminate themselves through the tank.

All fishes show this reaction in a greater or less degree. Most fishes, however, fail to respond after they have been kept in aquaria for some time and have become accustomed to the movements of people about the tanks. There is little question, I believe, but that this flight reflex is a response to stimuli through the action of the optic reflex apparatus described in this paper.

As noted by Edinger, the reflex is present before the fibre-tracts of the brain and cord are developed. This agrees with the conditions I have found in many fishes, where the cells of the apparatus are the first in the brain to become differentiated, and the optic reflex apparatus is fully established at the time of hatching, while the fibre-tracts of the brain are as yet undeveloped.

This flight reflex is early found in the larvae of all teleosts and amphibians. In the cyclostomes and ganoids, however, the apparatus is not fully developed until some days after hatching. There are published some data to show that during these days the larvae are sluggish and fail to respond to optical stimuli. I have had no opportunity to make observations with young cyclostomes or ganoids, but I am assured by a number of observers who have reared young Petromyzon that they are very sluggish during the early larval stages. From Dr. Mark, who has had a wide experience in raising the young of Lepidosteus, I learn that the same is true of that species. Of Amia, Reighard (:03) says, "When hatched, the larvae are about 7 millimetres long, colorless and incapable of progressive movement, and they either attach themselves to the nest material by the adhesive organ at the end of the snout, and remain suspended in a vertical position, or lie on their sides on the bottom. They remain in the nest for about nine days after hatching." Dr. Raymond Pearl of Michigan University, who has carried on extensive physiological experiments on the reactions of larval Amia to stimuli of various kinds, has kindly furnished me with the following data.

During the first day after hatching the young Amia stay on the bottom of the nest, exhibiting little movement except that of wriggling their tails. The second day they swim an inch or so, but the movements are not sufficiently co-ordinated to keep them moving any considerable distance. The third day they can swim a little farther, about four inches at one time. They cannot avoid obstacles, and after striking against them show a thigmotactic reaction, and drop to the bottom. The first ready response to optical stimuli comes at about the fifth day. By the sixth or seventh day they begin to swarm, and swim as older fish do.

In following the development of the optical reflex apparatus in Amia, we have seen that this apparatus is in process of development during the first ten days of larval life, and all the anatomical connections are established about the fifth day. This close correspondence of the anatomical development, with the development of activities, is almost conclusive evidence as to the connection of this apparatus with the flight reflex.

2. *Relation of the State of the Apparatus to Activity.*

Another line of evidence from the side of comparative physiology may be drawn from the relative development of the apparatus in species which are either very active or very sluggish. In any group, as, for example, the teleosts, the apparatus has its highest development in those animals which are most active. In the predatory and rapacious bluefish (Pomatomus), the apparatus is highly developed, Reissner's fibre having a diameter of ten micra. In Lophius, which is a much larger fish, but is sedentary in its habits, and more dependent upon tactile than visual sensations for obtaining its food, the apparatus is degenerate and Reissner's fibre inconspicuous.

3. *Effect of Degeneration of the Eye on the Apparatus.*

The effect of disuse of the eye, and the consequent degeneration of the optic reflex apparatus, is still more evident in those vertebrates which are blind or nearly so. For use in this study Prof. C. H. Eigenmann has kindly given me access to his extensive collection of preparations of cave vertebrates having degenerate eyes. Eigenmann (**'99**) has described the degenerate eyes of four species of Amblyopsidae, a group of small cave-inhabiting teleosts. In Chologaster papilliferus, in which the eyes, though functional, are much reduced, having a maximum diameter of 800 micra, I find a corresponding reduction in the optic reflex apparatus. Reissner's fibre is with difficulty distinguishable, having an approximate diameter of 0.5 micron. The torus is small, and in a state of arrested development. In the nearly related Fundulus, which is of the same size, and has normal eyes, the fibre has a diameter of one micron.

The three other species described by Eigenmann are totally blind and the whole optic apparatus, peripheral and central, degenerate. In Amblyopsis spelaeus, the vestigial eye has, according to Eigenmann (**'99**), a maximum diameter of 200 micra; in Typhlichthys, 180 micra; in Troglichthys, 85 micra. In the two latter species I have been unable to distinguish the slightest trace of Reissner's fibre. Ramsey (**:01**) has investigated the optic lobes and optic tracts of Amblyopsis, and finds them distinctly degenerate. The optic lobes are reduced greatly in size and the tectum is very thin (Fig. *G*). In Amblyopsis I find Reissner's fibre to be an exceedingly delicate filament which, in young specimens 6 to 10 mm. long, has a diameter of 0.1 micra. In larger specimens, 30 mm. long, the diameter was 0.8 mm.

In Amblyopsis the eye reaches a certain stage of development and then "with advancing age undergoes a distinct ontogenetic degeneration from the mature structure" (Eigenmann, **'99**). The presence of the rudiment of Reissner's fibre may be merely the persistence of a well-established structure, which in older individuals disappears; or it may be made up wholly of the axons which have their origin in the olfactory centres of the ganglia habenulae, and have been described by me in other species.

The Mauthner's fibres in Amblyopsis are of relatively large size, having a diameter about twice as great as in Fundulus. These fibres run from the medulla caudad through the cord, in which they lie one on either side of the median plane, ventral to the central canal. They constitute the conduction path for short-circuit transmission of motor reflexes from the *auditory* centres to the musculature. In the Amblyopsidae the sense of hearing has become, with the degeneration of the eye, more acute and the auditory reflexes have an increased importance, which has resulted in the greater size of Mauthner's fibres.

I have examined two other blind vertebrates. In Typhlotriton, a blind salamander, no evidence of Reissner's fibre was found. Rhineura, the blind lizard of Florida, has a very small Reissner's fibre. In specimens 28 to 30 cm. long, the diameter of the fibre was 0.8 micra, which is small for the size of the animal.

4. *Relation between the Optic Lobes and the Optic Reflex Apparatus.*

The corpora quadrigemina in higher vertebrates are degenerate organs, having given up most of their functions to other parts of the brain. "In lower forms the optic lobes — that is, the region of the corpora quadrigemina — are the main visual organs. In higher forms the corpora quadrigemina appear to be active mainly in reflex functions, while the lateral geniculate body represents the way station in the visual path to the occipital cortex" (Barker, **'99**, p. 804). "The superior colliculus of the corpora quadrigemina, so largely developed in lower animals, is but rudimentary in man" (ibid. p. 808). Since, then, the optic reflex apparatus has its centre in the superior colliculus, it must have, in the higher vertebrates at least, a purely reflex function.

5. *Relative Importance of the Visual Sense.*

In the lower vertebrates the relatively high development of the eye, as compared with the ear and other sense organs, naturally suggests that the visual sense is of the greatest importance in warning the animal of the presence of danger. This is probably especially true in amphibians and reptiles, in which there are no Mauthner's fibres as in fishes, and consequently no short circuit exists between the auditory centres and the musculature. Moreover, the sense of hearing is so little developed in the lower vertebrates that it cannot be relied upon to present promptly evidence of approaching danger. Parker (**03**, p. 186) has said, "The sense of hearing is probably the most recently acquired of the senses," and further, "though the frogs, toads, turtles, and their like have a sense of hearing, the efficiency of this sense is low compared with that which it attains in the birds, and particularly in the mammals, the highest vertebrates. It is thus evident that the sense of hearing is coupled with high organization." The observations of Yerkes (:**02**, p. 630) on the importance of the visual sense in the frog are of great interest in this connection. "One can approach to within a few feet of a green frog or bull frog and make all sorts of noises without causing it to give any sign of uneasiness. Just as soon, however, as a quick movement is made by the observer, the animal jumps. I have repeatedly crept up very close to frogs, keeping myself screened from them by bushes or trees, and made various sounds, but have never succeeded in scaring an animal into a motor response so long as I was invisible. Apparently they depend almost entirely upon vision for the avoidance of dangers. Sounds like the splash of a plunging frog, or the croak or pain-scream of another member of the species, serve as warnings, but the animals do not jump into the water until they see some sign or an unusual or dangerous object." The senses of hearing and smell give such inadequate warning of the presence of danger to these lower animals, that it is of the most vital importance that they should be able to react quickly to optical stimulus. This accounts for the greater development and greater importance of the optic reflex apparatus in the lower vertebrates, and its decreased value in mammals, where the sense of hearing and smell are more acute.

B. EXPERIMENTAL PHYSIOLOGY.

The desirability of physiological experiment to determine the function of this apparatus early presented itself to me. The only practical method of accomplishing this was to sever Reissner's fibre and study the reaction of the animal after the operation. In the great majority of animals this is practically impossible because of the difficulty of the operation and the great disturbances resulting from it, seriously impairing the validity of subsequent observations. Success is probable only in the lower vertebrates, where the fibre can be reached in the fourth ventricle through the thin tela chorioideus which covers it. To insure success, an animal should be chosen in which the brain flexures are slight, so that the ventricle is easily accessible from the surface. My first attempt was upon the alligator, which seemed to offer a favorable subject. My experiments, however, failed to yield any results because of the sluggishness of these animals and their slow response to optical stimuli. This may have been partly due to an abnormal condition resulting from long confinement and low temperature. Moreover, the operation was one of some difficulty, because of the necessity of cutting through the bony skull.

1. *Material.*

In the summer of 1900, while occupying at the U. S. Fish Commission at Wood's Hole one of the tables assigned by the Museum of Comparative Zoölogy, I had, through the courtesy of Dr. Bumpus, the director, the opportunity of conducting some successful experiments upon selachians. The animals used were of four species, the dusky shark (Carcharhinus obscurus), the sand shark (Charcharias littoralis), the spiny dog-fish (Squalus acanthias), and the smooth dog-fish (Mustelus canis). These animals are, for a number of reasons, particularly favorable subjects for this experiment.

2. *Method of Operation.*

In sharks Reissner's fibre is of relatively large size, from 8 to 20 micra in diameter, and in passing through the fourth ventricle into the central canal is at some distance above its floor. As a preliminary experiment, I took the brain and anterior portion of the spinal cord from a small shark, and attempted to draw out Reissner's fibre. Opening the fourth ventricle I took a fine needle, curved into a microscopic hook at its tip, and drew it transversely across the floor of the ventricle, until I had caught the fibre and could lift it free from the cerebro-spinal fluid. It was not until after many failures with successive brains that I was able to do this. I also succeeded in drawing the fibre out of the central canal. Taking a freshly cut section of the cord 1 to 2 inches long, I was able, after many trials, to seize the fibre with fine tweezers and draw it from the canal. The fibre when thus withdrawn is perfectly transparent, and many times finer than the finest hair, but may be readily seen in a strong light because of its high refractivity. It is somewhat elastic, contracting after being stretched. It has also a considerable degree of tenacity, so that in a number of cases I was able to stretch it across a glass slide. In this way I studied microscopically the freshly isolated fibre with the aid of methylen blue, and also made some permanent preparations of it.

The operation of cutting the fibre is simple and easy. A small shark three to four feet long was taken from the floating cage in which the animals were kept, and quickly placed on the operating table. By means of a rubber tube 1 to 2 inches in diameter, placed in its mouth and firmly held there, a stream of sea-water was kept flowing through the gills. In a few seconds the animal began to respire regularly, and ceasing its struggles, which were due to partial asphyxiation, remained quiet upon the table during the operation. If, however, the tube became displaced, or the flow of water was interrupted, it struggled violently. Pressure applied to the snout will call forth similar reactions. Aside from these regions, the shark seemed utterly devoid of any sense of feeling, and I have been unable to observe the slightest reaction to the knife. In the operation aseptic precautions were used as far as possible, though it is doubtful if they were efficient enough to be of value. All instruments and materials used were kept in a 1 to 2 per cent solution of carbolic acid. An incision, Z- or L-shaped, was made through the skin in a region previously determined upon, above the fourth ventricle. A triangular bit of cartilage was then removed, exposing the vascular tela. Through this a fine cambric needle, curved at the point as before described, was inserted and drawn several times transversely across the ventricle to insure breaking the fibre. At the point where the tela was punctured, there was a slight hemorrhage. The wound was then washed with 1 per cent carbolic acid, and closed with a cotton plug saturated in carbolated vaseline. The integument was then drawn together and closed by a number of sutures, and the wound rubbed over with vaseline to render it as nearly as possible waterproof.

The animal was then tagged for identification, and returned to the water, after having been out of water some ten to twenty minutes. Some of the sharks were put in 'The Pool,' a shallow enclosure 20 by 30 feet, others in the floating cage before-mentioned. Their behavior was closely watched for the first few hours, and observations made several times a day for a number of days, or until death ensued. Their general behavior and reactions to optical stimuli were compared with those of normal animals which had not been operated upon, but were confined in the same enclosure.

3. *Experiments.*

Six spiny dog-fish (Squalus), three smooth dog-fish (Mustelus), and two sand sharks (Charcharias) were experimented upon in this way. Squalus No. 1, during the first ten minutes after the operation, swam about sluggishly on its back, head downward, or rested upon the bottom. In half an hour it had partially, and in two hours wholly, recovered from the operation, and swam about quite normally, keeping near the bottom. It would bump hard against the side of the cage, as if that obstacle were not seen, and then follow the wall upward to the surface with its head curved dorsally. On the following day it swam about as actively and responded as quickly to tactile stimuli as its companions which had not been operated upon. When a stick or an oar was suddenly placed in front of it, the effort to avoid the obstacle came apparently too late to prevent collision.

Squalus Nos. 2, 3, and 4 recovered more quickly from the shock of the operation than No. 1 did. In No. 4 there was scarcely any apparent shock. Compared with normal individuals, these three showed the same slowness of response to optical stimuli as did No. 1. On the third day all these individuals became sluggish and died on the fourth or fifth day. Autopsies performed on all of them showed the tela more or less torn and congested. The central nervous system was not otherwise injured. There was every evidence that the fibre had been broken, but the brain and anterior portion of the cord were preserved for microscopical examination to determine this absolutely. Death was caused by diffuse meningitis, both spinal and cerebral. In teased specimens of the dura mater numerous bacteria, cocci, and bacilli were present. The membranes were deeply injected and the wounds more or less ulcerated.[1] Had antiseptic and waterproof dressing been devised for these animals, it seems probable that they would have recovered from the operation, though it is true that sharks have shown almost no capacity to heal wounds or regenerate skin. Liquid rubber, collodion, and other dressings were used, but were less satisfactory than vaseline.

Squalus No. 5 gave evidence of severe shock after the operation, probably because it was abnormal at the time, having been confined for some months previously. It, however, showed the normal response to optical stimuli, not the slow response observed in the previous cases. In Squalus No. 6 the same operation was performed, but care was taken not to injure Reissner's fibre. After recovery from shock, it responded quite normally to optical stimuli.

Of the three specimens of Mustelus operated on, two gave substantially the same results as Squalus No. 1; the third, the same as Squalus No. 5. Two sand sharks (Charcharias) were operated on, but the results were unsatisfactory. There was no recovery from shock, and no characteristic reactions could be observed.

The great difficulty in carrying on these experiments with water animals is in preventing infection and consequent meningitis. To make these experiments conclusive, the time reaction to optical stimuli should be taken both before and after the operation, and also after complete recovery.

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[1] For assistance in performing these autopsies I am indebted to Dr. H. H. Cushing, of Jefferson Medical College.

These experiments, though incomplete, show clearly, I believe, that when Reissner's fibre is severed the power to respond quickly to optical stimuli is lost.

C. PHYSIOLOGICAL VALUE OF THE APPARATUS.

We are now naturally led to the consideration of the value of this apparatus in the life activities of the animal. We have seen something of how, as an archaic element of the nervous system, the more complex structures have been built up about it. It probably existed in a form like the single median giant fibre connected with the pigment spot of Amphioxus before the central nerve-paths of the cord were well developed. But not satisfied merely with throwing some light on its origin, we must now attempt to explain why this unusual structure should persist throughout the vertebrate series. We are logically led to suppose that it is of some advantage to the animal, and the most probable advantage is that it offers a 'short circuit' for the transmission of motor reflexes, by which a reaction may be brought about more quickly than when the impulse is transmitted through the nerve-tracts of the spinal cord.

This leads us to the consideration of the time relations involved in these central processes. The data at hand for a comparison of the time of transmission of nervous impulses along different paths in the nervous system are as yet meagre. Sufficient reliable data exist, however, to show that in the reflex response to optical stimuli through the apparatus connected with the fibre of Reissner, there are at least two sources of time-saving: (1) in the rate of transmission through the conducting substance; (2) in the simplicity of the central changes in the optic lobes, where, by a reduction in the number of neurons involved, there is avoidance of delay in the passage through the finer arborizations from neuron to neuron, and through the cell bodies of nerve-cells.

1. *Rate of Transmission of the Nervous Impulse.*

The rate of transmission of the nervous impulse was first measured by Helmholtz ('50), who found it in the sciatic nerve of the frog to be about 27 m. per second under normal conditions. He afterward determined the rate in man to be about 30 m. per second in both the sensory and motor nerves.

Since Helmholtz's work other more accurate and detailed investigations of the rate of nerve transmission have been made, but his figures still stand as approximately correct. Dolley and Cattell (**'94**) found it in man to vary in individuals from 21 to 49 m. per second. In the average man it is probably about 34 m. per second under normal conditions, reaching as high as 90 m. at high temperature and in exceptional cases.

The rate varies in different animals, and directly with the complexity of the organism. In lower animals it is much slower. In the medullated electric nerve of the torpedo it is from 8 to 20 m. per second, and in Malapterurus, 28 m. per second. Moreover, it is now known that the rate varies for different nerves in the same animal, and for the same nerve from day to day, or with changes in the physical conditions, temperature, etc.

There is, too, a relation not generally recognized between the rate of transmission of the nervous impulse and the degree of insulation of the nerve-fibres. In those nerves which are insulated by a well-developed medullary sheath, the rate is most rapid. Chauveau (**'78**) carried out a series of observations on the horse to determine the relative rate of transmission in the motor nerves supplying the voluntary muscles of the larynx, and those supplying the involuntary muscles of the oesophagus. He found that in the non-medullated nerves to the involuntary muscles the rate of transmission is at most 8 m. per second, or nearly four times as slow as that of the medullated nerves to the voluntary muscles.

The rate of transmission in the non-medullated nerves of invertebrates is still slower. In the nerve for the claw muscles of the lobster, Fredericq et Vandevelde (**'79**, **'81**) found the rate to vary with the temperature from 6 to 12 m. per second. Jenkins and Carlson (**:03**) found the rate of transmission in the ventral nerve-cord of worms to vary from 5 to 9 cm. per second in nemerteans, to from 54 to 694 cm. in annelids. According to Fuchs (**'94**), the rate of nerve-transmission in the non-medullated nerves of the cephalopod Eledone moschatus during the winter is about one metre per second. Boruttau (**'97**) has found it, during the summer, to be from 3.5 to 5.5 metres in both Eledone and Octopus. In the nerves of the mantle of Eledone, the rate was found by Uexküll (**'94**) to be from only 0.4 to 1 m. per second, and in Anodon, according to Fick (**'63**), it is as low as one centimetre per second.

Aside from the observations of Chauveau, there have not been, to my knowledge, any other investigations on the relative rate of transmission in medullated and non-medullated nerve in the same animal. Other physiological differences between these two kinds of nerve have, however, been brought out by Kühne und Steiner ('79). Having demonstrated the resting current in a suitably prepared olfactory nerve of a fish, they proceeded to compare the value of this current with that obtained from medullated nerve of the same diameter in the same fish, as well as from the sciatic nerve of the frog. The result showed a much higher electromotive force for the non-medullated olfactory nerve. This result has been confirmed by Biedermann ('86) and others, working on the non-medullated nerves of Anodonta, and by Sowton (:00), on fishes. Sowton (:00) found still other physiological differences between medullated and non-medullated nerve. "Whereas in medullated nerve successive effects diminish very slightly, or not at all, or actually increase ('staircase effect'), non-medullated nerve always exhibits a comparatively rapid decrease of successive effects." . . . "Isolated gray nerve appears to be far less resistant than white nerve."

I have elsewhere shown (Sargent, :04) that the medullary sheath of the nerve-fibre has for its principal function the insulation of the separate nerve-fibres.

There are, then, considerable physiological differences between medullated and non-medullated nerve-fibres, and especially in the rate of transmission of the nervous impulse. In those fibres which are well insulated, the transmitting impulse is conserved and the rate of transmission is more rapid. The conducting substance in the fibre of Reissner is not only surrounded by a medullated sheath, but it is also isolated from the other conduction paths of the nervous system, and further insulated by the surrounding cerebro-spinal fluid. If, then, the degree of insulation so directly affects the rate of transmission of the nervous impulse, it is at least probable that in the fibre of Reissner transmission is more rapid than in other conduction paths of the same nervous system. It seems, indeed, not improbable that in this separated and highly specialized conduction path the rate of transmission may be several times as fast as through the ordinary axis-cylinder.

2. *Time Relations of Central Processes.*

As is well known, the period which necessarily elapses between the presentation of a stimulus and the time at which a signal of recognition is given by the observer is known as the 'physiological time.' This varies for different stimuli and for different observers. The individual variability is so important in astronomical observations that it is necessary to consider the 'personal equation' of different astronomers in comparing their observations.

Many observations have been made of the physiological time in the case of different stimuli. Kries und Auerbach (**'77**), as the result of eight series of investigations on the human subject, give the following figures as most probably correct: for optical stimuli, 0.193 sec., for acoustic stimuli, 0.120 sec., for touch stimuli, 0.117 sec. It will be noticed that the simplest reaction-time can, under the most favorable conditions, scarcely be reduced to 0.1 sec., while it rarely rises much above 0.2 sec. The reaction-time for acoustic and touch sensations is nearly the same, but that for optical sensations is nearly twice as great.

It has been argued that the apparent differences in the reaction-times of the different senses is due to difference in the intensity of the stimuli applied, for an increase in the strength of the stimulus does decrease the reaction-time in any one of the senses; but according to Ladd (**'97**): "There seems good reason to suppose that the reaction-time of sight is necessarily longer than that of hearing or touch, on account of the photo-chemical nature of its more immediate stimulus. One observer (von Witlich) has even gone so far as to conjecture that the speed of conduction in the optic nerve is less than that of the other nerves of sense; it is rather to be concluded, however, that the latent time of the sensory end apparatus, and of the cerebral processes by which sensory impulses pass over into motor impulses, is different."

The deliberate method of experiment used in determining the physiological time with reference to optical stimuli, is not of the sort to call into action the short-circuit optic reflex apparatus. As there is no element of surprise or danger, the stimulus is probably not intense enough to bring about the shorter reaction, as would be the case in dodging or guarding, in boxing or fencing. The fact that such deliberate reactions to optical stimuli take so much longer than similar reactions to auditory or touch stimuli, we may conjecture to be due to the fact that the usual conduction path (*i. e.* the chain of neurons involved) for such reactions to optical stimuli is less highly perfected than the similar conduction paths for acoustic and touch stimuli. The reason for this may be that there has been no necessity for such perfection, because from the earliest phylogenetic time there has been this short-circuit optic reflex apparatus to be relied upon in cases of emergency. This, it is true, is merely a speculative suggestion as to a correlation of causes.

But if the optical reflex function of this apparatus is admitted, the above statements then become corollary truths.

3. *Delay in the Conduction Path.*

The conduction path of every nervous impulse is formed of a chain of neurons. In the simplest reflex processes this may consist of only two neurons, one sensory and the other motor, in which case there would be but one place of juncture, that between the cell processes of the sensory cell and the dendrites or cell body of the motor cell. In the more complex nervous reactions, on the other hand, the impulse must be transmitted over a chain of several neurons, with a synapse at the place of contact between each two links of the chain. We have as yet insufficient data from which to ascertain how the delay which accompanies the transmission through such a chain of neurons "is to be apportioned between the fine nerve-endings, the unknown field of conjunction, and the branches of the efferent cell through which it is discharged into the issuing efferent fibres. It is logical to assume that in each one of these three situations the difference of structure involves a corresponding difference in the rate of propagation, and that the sum of central delay is the sum of the retardation in all of these situations" (Gotch, :**00**, p. 483).

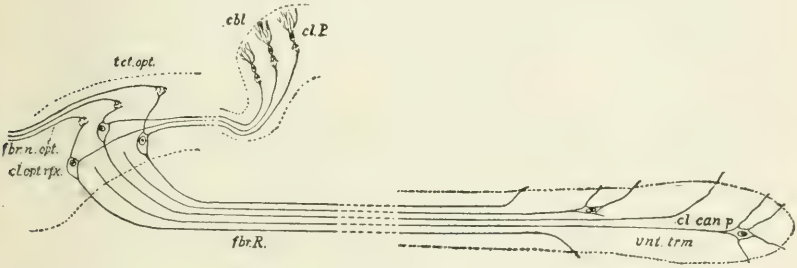

FIGURE *M.* Diagram of the optic reflex apparatus. For meaning of abbreviations, see Explanation of Plates, p. 257.

"There is reason to believe," says Schäfer (:**00**, p. 608), "that the additional delay ('lost time') which is characteristic of the passage of nervous impulses through the nerve-centres, is due to a block at each synapse; that, in fact, the nerve-impulses are momentarily arrested at these places of contact of the nerve-cells with one another. And it is not improbable that the relative number of these blocks will furnish a key to the differences which are found to obtain in the reaction time for different reflexes and psychical processes."

In the following pages I have attempted to make use of the fragmentary data which exist, in estimating the time of an optical reflex conducted through the usual paths of the spinal cord. I then attempt a comparison of this time with the time necessary to conduct an optic reflex over Reissner's fibre. In doing this I lay myself open to criticism for introducing estimates which are subject to so large a percentage of error. But the method I believe promises much, and a beginning must be made somewhere.

a. Central Delay.

Wundt (**'76**) found that the reflex time in the frog, when stimulated at the lower part of the spinal cord, was 0.008 second less than when the sensory cells were stimulated; that is, 0.008 second was the time occupied by the passage of the impulse through the central nervous system. In the case of a crossed reflex this was increased 0.004 second, that is, to 0.012 second. Now if this crossed reflex was produced by the interposition of a single neuron, then the delay due to one synapse would be 0.004 second, but if by two neurons, it would be only 0.002 second. Assuming the first conjecture to be correct, the reflex in its transmission through the brain and cord must pass through a chain of (0.012 ÷ 0.004) three neurons.

Exner (**'74**) found that the reduced reflex time of the closure of the eyelid on direct excitation was 0.047 second, hence this more complex reaction must involve a chain of many more neurons than in the preceding case. He also found that the 'lost time' between excitation of the frog's cerebrum and the response of the leg muscles is 0.05 second. In the cerebrum and optic lobes about 0.025 second is lost, between the bulb and the lumbar enlargement only about 0.015 second, and in the sciatic nerve and muscle plate still less. Exner concludes, as the result of many experiments, that the time of block in a synapse of the brain may be 0.008 second. This time is accepted by Schäfer (**:00**) as a reliable working value. Gotch (**:00**, p. 483) says "the central delay for apparent ending in the spinal cord is at least 0.006 second and may be considerably more;" and he had previously (**'87**, p. 510) shown that this delay must be regarded as due to retarded transmission in the arborizations.

Langendorff und Krawzoff (**'79**), working with the frog by electric stimulation and the graphic method, found that stimulation of the cerebrum resulted in a contraction of the leg muscles 0.036 second later than when the electrodes were applied directly to the sciatic nerve. If, instead of the cerebral hemispheres, the cut surface of the medulla spinalis was stimulated after its severance from the medulla oblongata, the delay is only 0.017 second greater than when the sciatic nerve is stimulated. They therefore concluded that the delay between the cerebrum and the cord is about 0.020 second. The whole time lost between the surface of the brain and the gastrocnemius muscle is 0.050 second. Of this, 0.020 second occurs in the brain, 0.015 second in the cord, and the rest in the nerve and muscle.

Of much greater value in this discussion are the results of Wilson ('**90**, p. 504). He found that by direct stimulation of the optic lobe of the frog from which the cerebrum was removed the m. triceps femoris reacted in 0.044 second. When the spinal cord was stimulated at its anterior end, the reaction time was 0.020 second, showing a delay of 0.024 second in the passage of the impulse from the optic lobe to the cord, which according to Wundt's estimate would indicate six synapses in the conduction path.

The results of Exner, Langendorff und Krawzoff, and Wilson agree fairly well. They show a delay in the brain proper of respectively, 0.025, 0.020, and 0.024 second; and a delay in the cord of approximately 0.015, 0.017, 0.020— second.

Similar reaction-time experiments have been made on the dog by François-Franck ('**77**), Pitres ('**83**), Bubnoff und Heidenhain ('**81**), Novi e Grandis ('**87**), and Fano ('**95**). These investigators find that the delay of the nerve-impulse in the cerebral cortex is from 0.045 to 0.020 second, or a little less than in the frog. Fano found that the extirpation of the motor zone of the cortex causes a shortening of the reaction time, whereas electric stimulation of the cortex results in a lengthening of the reaction-time. From his experiments Fano concludes that the ganglion cells of the cortex perform an inhibitory function. Schäfer ('**88**) got results with the monkey similar to the above.

It will be seen that the results of Exner, of Langendorff und Krawzoff, and of Wilson on the frog are in substantial agreement, and may be briefly summarized as follows: The time which necessarily elapses between the stimulation of the brain surface and the contraction of the leg muscles is about 0.050 second. Half of this delay, 0.025 second, occurs in the brain, about 0.015 second in the cord, and the remainder, 0.010 second, in the sciatic nerve and muscle. Bernstein ('**82**) finds the time lost in the muscle-plate to be 0.0032 second. If we allow 10 cm. for the length of the sciatic nerve in the frog, the delay in transmission would be 0.0033 second, leaving a remainder of 0.0035 second for the latent time of muscular contraction.

The results of the above investigations of physiological time do not reveal the normal reaction-time. The physiologists, in studying the time relations of neural processes, have begun from within, and by stimulating the different parts of the brain have attempted to learn how the time occupied in efferent transmission is distributed among the brain regions. The psychologists, on the other hand, have attempted to measure the normal reaction-time to stimuli of peripheral sense-organs. The results of the physiological method permit of a further time analysis, as I have shown above; the results of the psychologists cannot be so analyzed. Much work has been done by the psychologists in determining the reaction-time of man, but practically nothing has been done on the lower animals. Yerkes (:**02**) has, however, done valuable work of this kind on the frog. He finds the normal reaction-time of the frog to tactual stimuli to be about 0.2 second, or about twice as great as in man. The reaction-time to comparable electrical stimuli is somewhat less. He was unable to measure the reaction-time to optical stimuli.

b. DELAY IN TRANSMISSION THROUGH THE CELL BODY.

It has been conclusively shown by S. Ramon y Cajal and by Bethe ('**97**) that in some nervous processes the impulse is not necessarily transmitted through the cell body. Bethe ('**97**) has proved in Carcinus that transmission may even take place normally when the fibres have been severed from their ganglion cells. But it is doubtless equally true that in the great majority of central processes the nervous impulse is transmitted through the perikaryon. It is commonly assumed that in the transmission of a nervous impulse through the cell body, the impulse is delayed, though the evidence on this point is not wholly conclusive. Wundt ('**76**, Abth. **2**, p. 45) found a delay of 0.02 second in transmission through the spinal ganglion of the frog. Doubt has been thrown on this result by Exner ('**77**, p. 567), who has pointed out that the nerve-impulse, in passing through the posterior roots, is not transmitted through the cell body, as Wundt supposed; and Moore and Reynolds ('**98**, p. 56), repeating Wundt's experiments, have shown that when in two experiments the path of the impulse through the cord was the same, the interposition of the ganglion caused no appreciable delay. When, however, the impulse is obliged to traverse the cell body, a delay occurs. Gad ('**89**) showed "that the interposition of the vagus ganglion caused a retardation of 0.03 to 0.04 second in the respiratory-centre inhibition, which is produced by excitation of the vagus trunk."

The evidence obtained by reaction-times as to the number of synapses traversed by a motor reflex to the peripheral nerves, though reliable only within wide limits, shows that the simpler motor reflexes involve transmission through a chain of from three to six neurons and consume a considerable time, — from 0.03 to 0.05 second.

Our morphological knowledge of the chains of neurons involved in such processes as the last is not sufficiently exact to enable us to tell just how many neurons are included in this chain, but there is reason to believe that there are at least three, for even the simpler reflexes.

4. *Time of Optic Reflex Reaction.*

We have seen that the average time of delay at a synapse in the central nervous system may be conservatively estimated at 0.006 second. The delay in transmission through a cell body cannot be considered as known, nor do we know in how many of the neurons the impulse passes through the cell body. We may accept it as determined that the impulse is in some cases transmitted through the cell, and that in such cases there is a delay. Taking three neurons as a conservative estimate of the number in the chain by which ordinary reflexes in the frog (the animal in which these values have been best determined) are transmitted from the ending of the optic fibres to the musculature, we may estimate the time involved in transmission, and compare it with the probable time over the short circuit path of Reissner's fibre. The time of a reflex through the cord may then be estimated as follows: three central synapses ($0.006 \times 3 = 0.018$), plus muscle-plate arborization (0.003), plus transmission over 0.2 m. nerve (0.007), plus delay in two nerve-cell bodies ($2\,x$) gives for the total time $0.028 + 2\,x$ second. Subtracting from this the time of peripheral delay, we have approximately $0.025 + x$ second, which corresponds favorably with Wilson's result of 0.024 second for central delay in the frog.

5. *Time saved by the Optic Reflex Apparatus.*

In the transmission of a reflex over the Reissner's-fibre path a single neuron suffices to bridge the gap (Fig. *M*), so that but two synapses and one cell body must be traversed. Moreover, the rate of transmission along the conducting substance may, as we have seen, be safely taken as twice as great as ordinary transmission, giving the following estimate of time: one central synapse (0.006), plus muscle-plate arborization (0.003), plus transmission time (0.003), plus delay in one cell body (x) gives a total time of $0.012 + x$ second. This is $0.016 + x$ second less than by the path through the cord, or less than one-half. Any such estimate is of course subject to a large error because of the inaccuracy of the data on which it rests; however, the comparison suffices to show that the Reissner's-fibre apparatus may be a great time-saver. It is evident that, in the lower vertebrates particularly, this short circuit may mean the saving of a considerable fraction of a second.

In the struggle for existence an animal may be frequently presented with ocular evidence of danger, the only safety from which lies in flight, dodging, or some other quick reaction from the source of danger. A decrease of a small fraction of a second in the reaction-time may often be a matter of escape or injury, of life or death, and so the determining factor in the survival of the fittest individual or species. The optic reflex apparatus, then, not only plays an important part in the life of the individual, but its functioning has probably often been a determining factor in the evolution of vertebrates.

The conclusions and the discussion of the results and bearings of this research are reserved for the second part of this paper, dealing with the higher vertebrates. This is already well advanced, and it is hoped will appear in about a year.

June, 1904.

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EXPLANATION OF THE PLATES.

Except where otherwise stated, all the figures are oriented so that the dorsal side is uppermost, and the anterior end at the left. All the figures are drawings from single sections, except where the contrary is noted. Each drawing was outlined with an Abbé camera lucida.

ABBREVIATIONS.

ac.	acusticum.
ax.	axon.
ax.'	axon to opticus.
ax.''	axon to tractus tecto-cerebellaris.
ax.'''	axon to tractus a tecto ad fibrae Reissneris.
ax. opt. rfx.	axons of the optic reflex cells.
ba. pdl. e'phy.	base of epiphysial stalk.
can. c.	canalis centralis.
can. n'enr.	neurenteric canal.
cbl.	cerebellum.
cd. d.	chorda dorsalis.
cd. spi.	spinal cord.
cl. can. p.	posterior canal-cells.
cl. d.	Median dorsal cells of the spinal cord ('Hinterzellen').
cl. e'end.	ependymal cells.
cl. g.	germinal cells ('Keimzellen').
cl. n'gli.	neuroglia cells.
cl. opt. rfx.	optic reflex cells.
cl. Purk.	Purkinje cells.
cl. tct. rfx.	tectal reflex cells.
co'ms. p.	commissura posterior.
co'ms. IV.	commissura trochlearis.
cps. pi'cl.	pericellular capsule of neuroglia fibres.
cras. e'end.	ependymal thickening (crassamentum).
crnu. l.	lateral horn of the mesencephalic recess, above the posterior commissure.
dec.	partial decussation of the tractus tecto-cerebellaris.
dec. d.	dorsal decussation of the tectum opticum.
dec. e'phy.	epiphysial decussation.
di'cœl.	diacoele.
dnd.	dendrites.
e'end.	ependyma.
e'phy.	epiphysis.
fas. Mey.	Meynert's bundle.
fas. Reis.	fasciculi of axons which unite to form Reissner's fibre.
fbr. e'end.	ependymal fibres.
fbr. hab. Reis.	habenular elements of Reissner's fibre.
fbr. n'gli.	neuroglia fibres.
fbr. Reis.	Reissner's fibre.
fis. i'tor.	median ventral fissure between the lateral lobes of the torus longitudinalis.
fnd. e'end.	fundament of the ependymal thickening.
gn. hab. dx.	ganglion habenula dextra.
gn. hab. s.	ganglion habenula sinistra.
gran. pig.	pigment granules.
h'phy.	hypophysis.
ifb.	infundibulum.
la. hyl.	hyaline layer.
lim. ex.	limitans externa.
lim. i.	limitans interna.
ms'cœl.	mesocoele.

ms'ence.	mesencephalon.
n'bl.	neuroblasts.
nidl. tect.	nidulus tectalis ('Dachkern').
nidl. III.	nidulus of III. nerve.
nl.	nucleus.
pa'phy.	paraphysis.
pdl. e'phy.	epiphysial stalk.
pdl. pin.	pineal stalk.
plx. chd.	plexus chorioideus.
prs. i'cal.	pars intercalatus ('Schaltstück').
rec. l.'	horn-like prolongation of the right lateral recess.
rec. ms'coel.	mesocoelic recess above posterior commissure.
rec. sb. pin.	recessus sub-pinealis.
rm. Reis.	branches of Reissner's fibre entering the walls of the canal.
rx. III.	root of III. nerve.
sac. e'phy.	epiphysial sac.
st. med.	stratum medullare profundum.
sul. l. e'end.	lateral ependymal groove.
sul. m.	median sulcus.
tct. opt.	tectum opticum.
tor. lg.	torus longitudinalis.
tor. si'crc.	torus semicircularis.
trt. opt.	tractus opticus.
trt. tct. cbl.	tractus tecto-cerebellaris.
trt. tct. fbr. Reis.	tractus a tecto ad fibrae Reissneris.
trt. tor. cbl.	tractus toro-cerebellaris.
trt. tor. fbr. Reis. a.	tractus a toro ad fibrae Reissneris anterior.
trt. tor. fbr. Reis. p.	tractus a toro ad fibrae Reissneris posterior.
trt. tor. tct.	tractus toro-tectalis.
tu. med.	medullary sheath (tunica medullaris).
va. sng.	blood-vessel.
vlv. cbl.	valvula cerebelli.
vrg. sb'cd.	sub-chordal rod.
vsl. trm.	vesiculus (or ventriculus) terminalis.

PLATE 1.

Fig. 1. Petromyzon planeri, 26th day. Parasagittal section (anterior at right, a little to the right of median plane) of the anterior portion of the roof of the mesencephalon, showing the optic reflex cells with their axons protruding into the ventricle. × 900 ca.

Fig. 2. Petromyzon planeri, 30th day. Parasagittal section (anterior at right, a little to one side of the median plane) of the anterior portion of the mesencephalic roof, showing the optic reflex cells with their axons passing into the mesocoele. × 800 ca.

Fig. 3. Petromyzon planeri, 30th day. Transverse section, a little caudad of the posterior commissure, showing the median nidulus of optic reflex cells. × 900 ca.

Fig. 4. Petromyzon planeri, 30th day. Transverse section through the diencephalon and the ganglia habenulae. Viewed from in front. × 205.

Fig. 5. Petromyzon marinus, adult, 30 cm. Parasagittal section through the roof of diencephalon and base of right ganglion habenula, showing the habenular elements of Reissner's fibre entering the diacoele. × 700 ca.

Fig. 6. Petromyzon marinus, adult. Transverse section through the right lateral ventricular groove, and horn, showing the axons of the optic reflex cells passing through the ependymal thickening, entering the ventricle and uniting in fasciculi. × 700 ca.

Fig. 7. Petromyzon marinus, adult, 30 cm. Transverse section through the anterior portion of the mesencephalon and posterior commissure, showing the lateral ventricular grooves with their ependymal thickening, and a few of the large optic reflex cells. × 35.

Fig. 8. Petromyzon marinus, adult. Sagittal section of the posterior portion of the spinal cord through the ventriculus terminalis, within which is the contracted and convoluted Reissner's fibre. × 385.

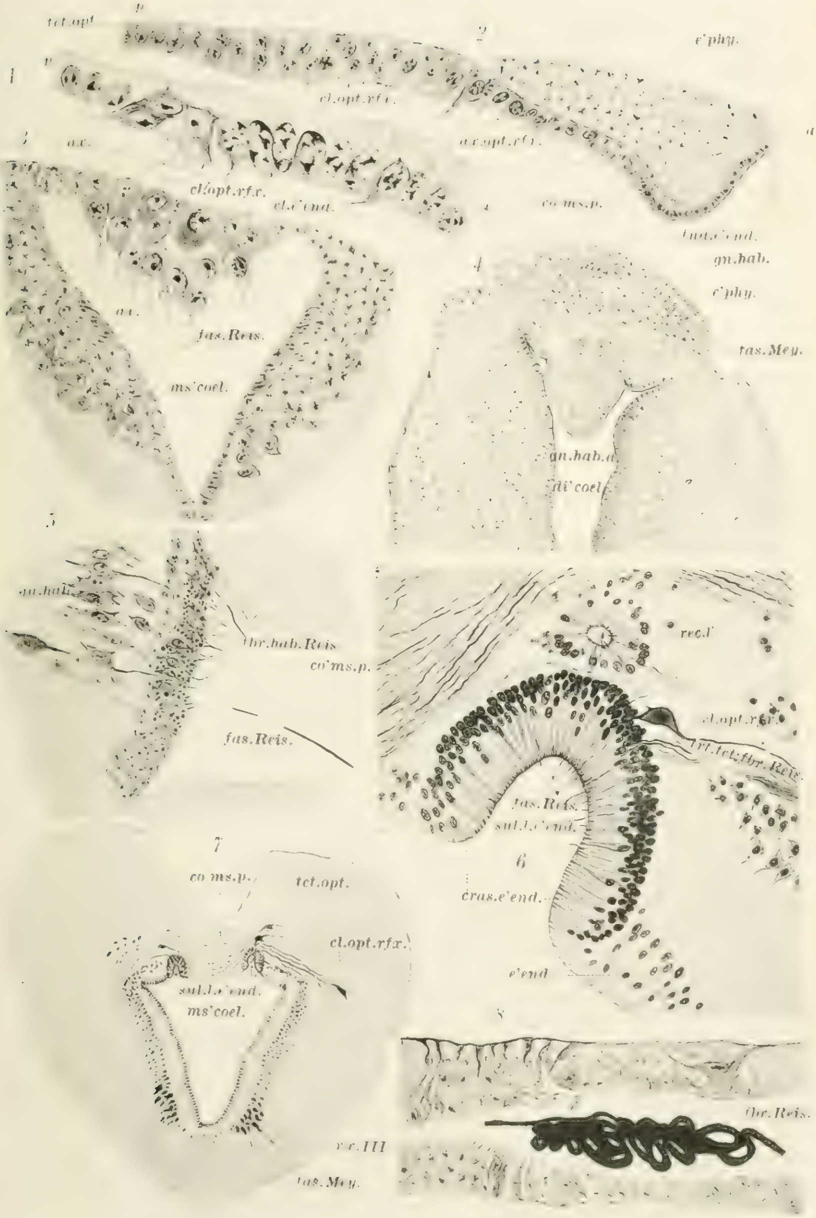
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PLATE 2.

Fig. 9. Mustelus canis, adult. A sagittal section of the midbrain roof, showing the 'Dachkern,' and anteriorly a portion of Reissner's fibre within the ependymal groove. The anterior portion of this section is strictly median; the posterior portion is somewhat lateral of the median plane. × 15.

Fig. 10. Mustelus canis, adult, same series. Parasagittal section (500 micra to the right of the preceding figure) of the adjacent portions of the roof of the diencephalon and mesencephalon. The section passes through the posterior commissure and right ganglion habenula, and cuts the lower lip of the ependymal groove, within which is Reissner's fibre. × 30.

Fig. 11. Mustelus canis, adult, same series. Parasagittal section through the trochlearis commissure and adjacent portions of the tectum opticum and cerebellum. × 45.

Fig. 12. Raja erinacea, at time of hatching, 12 cm. Transverse section through the middle portion of the mesencephalon and the 'Dachkern.' × 25.

Fig. 13. Squalus acanthias, 'pup,' at time of birth. Oblique parasagittal section through the diencephalic roof and anterior portion of mesencephalic roof. (The lower part of the figure is more nearly median than the upper portion, to show the course of the tractus tecto-fibrae Reissneris.) × 40.

Fig. 14. Mustelus canis, adult. Transverse section through the ependymal groove and posterior commissure. × 30.

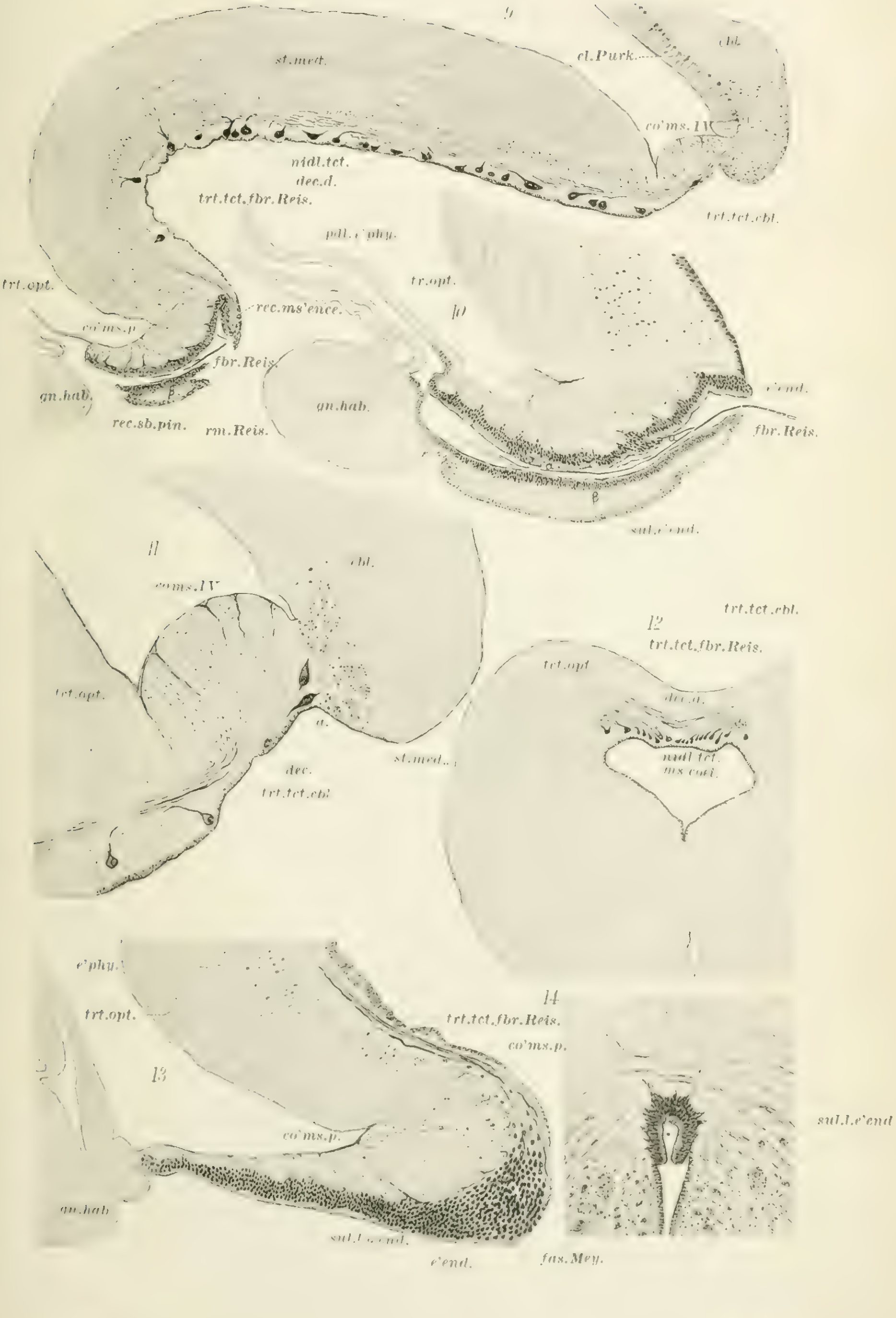
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PLATE 3.

Fig. 15. Raja erinacea, adult. Parasagittal section of the midbrain roof. The section is somewhat oblique, the anterior portion of the figure being nearer the median plane than the posterior portion. × 40.

Fig. 16. Raja erinacea, adult, same series. Sagittal section through the ependymal groove, posterior commissure, and right ganglion habenula. (Compare Fig. 10.) × 50.

Fig. 17. Squalus acanthias, embryo, 4 cm. long. Sagittal section of the brain. × 15.

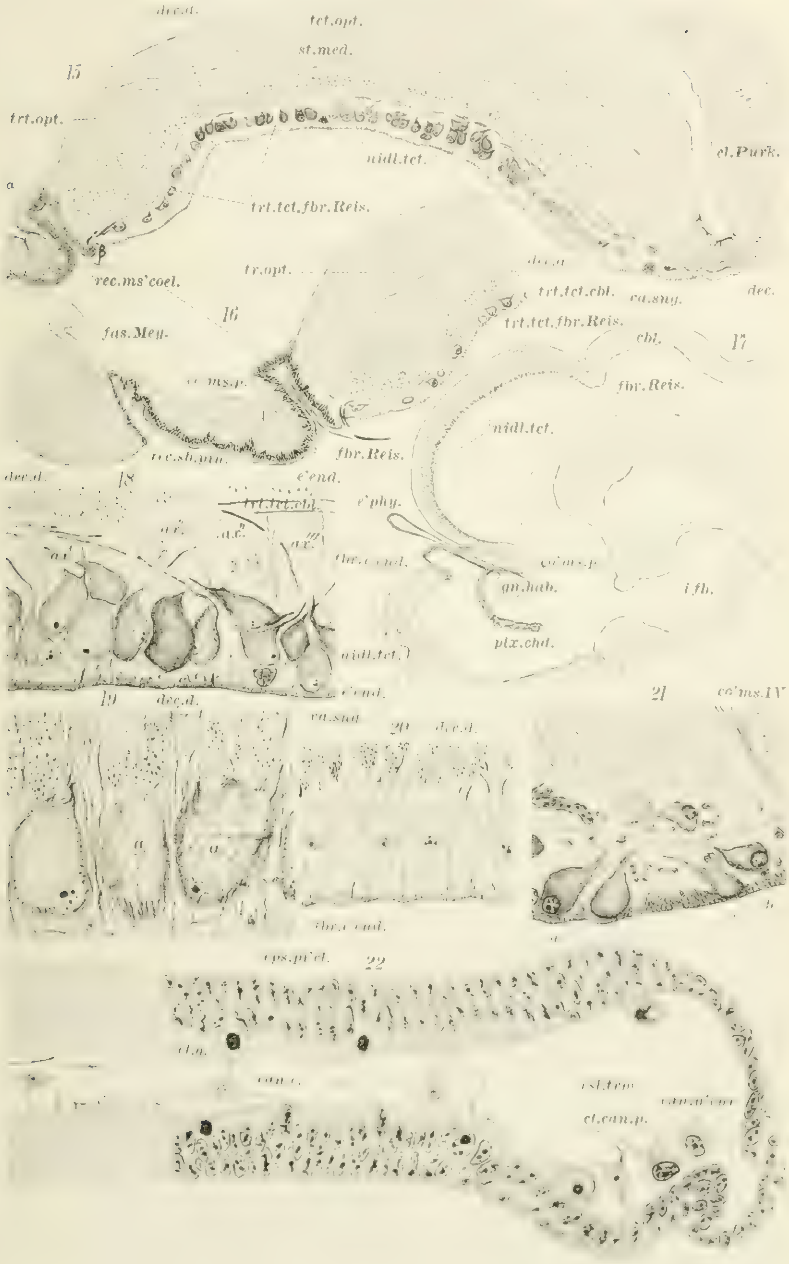
Fig. 18. Raja erinacea, young, 16 cm. Parasagittal section of the middle portion of the roof of the mesencephalon, through the 'Dachkern.' Details from three adjacent sections. × 160.

Fig. 19. Raja erinacea, adult. Parasagittal section through the 'Dachkern.' The cell at the left is shown in section, the two at the right show the outer surface in perspective, with anastomosing arborizations of nerve-endings. The pericellular neuroglia capsule is cut tangentially. × 240.

Fig. 20. Raja erinacea, adult. Sagittal section through the posterior portion of the 'Dachkern.' × 80.

Fig. 21. Mustelus canis, adult. Parasagittal section through the posterior portion of the mesencephalic roof, close to the cerebellum, showing the immediate proximity of the 'Dachkern' cells to the ventricle. × 80.

Fig. 22. Squalus acanthias, embryo, 4 cm. long. Sagittal section of the posterior end of the cord and ventriculus terminalis. The posterior canal-cells have been filled in from four adjacent sections, representing the cells projected on the median plane. × 385.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dec.d.
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dec.d.
21
co'ms.IV
a
a
fbr.c.end.
cps.pi'el.
22
cl.n.
cl.can.p.

PLATE 4.

Fig. 23. Amia calva, just before hatching. Sagittal section, anterior at right, through the roof of the midbrain, showing the primitive flexure in the midbrain roof, and the optic reflex cells in an early stage of differentiation. × 120.

Fig. 24. Amia calva, 1st day after hatching. Sagittal section, anterior at right, through the anterior portion of the optic tectum, posterior commissure and epiphysis. × 120.

Fig. 25. Amia calva, 3d day. Sagittal section, anterior at left, through the posterior commissure and anterior portion of the optic tectum. A number of the optic reflex cells are shown, and axons of other similar cells are seen entering the ventricle, and uniting. The fine divisions of the fibre of Reissner have been drawn in from two adjacent sections. × 200.

Fig. 26. Amia calva, 6th day. Parasagittal section, anterior at left, of the same region as the above. × 400.

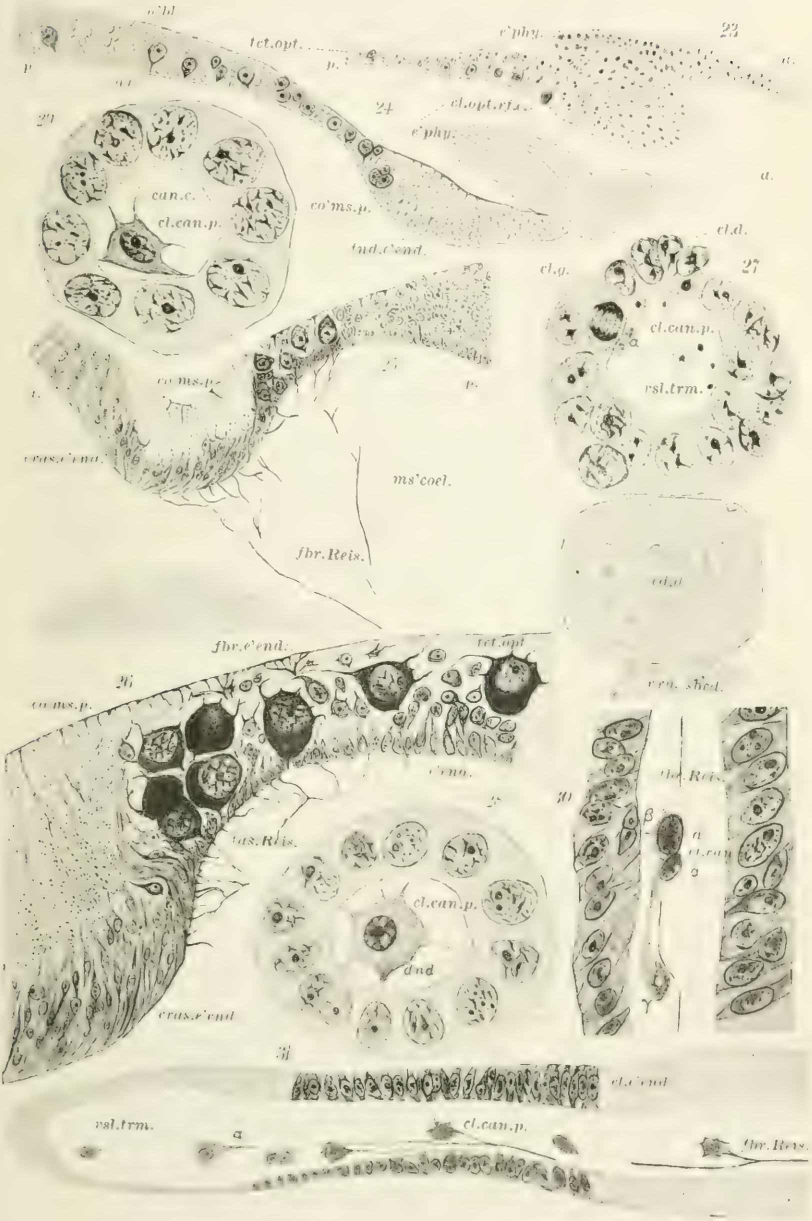
Fig. 27. Amia calva, 1st day. Transverse section of the cord and ventriculus terminalis, showing, within the lumen and in process of dehiscence from the wall of the cord, the cells which form the posterior canal-cells. (The contents of the lumen have been filled in from two adjacent sections.) × 1200.

Fig. 28. Amia calva, 6th day. Transverse section through the ventriculus terminalis. A single posterior canal-cell nearly filling the lumen of ventriculus. × 1200.

Fig. 29. Transverse section, same series, 30 μ anterior to the preceding. × 1200.

Fig. 30. Amia calva, 2d day. Sagittal section through the posterior portion of the cord. The anterior end is uppermost, ventral at the left. Two posterior canal-cells, α, α, recently given off from the ventral wall of the canal, and in process of differentiation, are shown, connected with a third cell, γ, by a protoplasmic strand. × 900.

Fig. 31. Amia calva, 6th day. Sagittal section of the posterior extremity of the cord and ventriculus terminalis. The posterior canal-cells and their axons have been drawn in from three adjacent sections. × 385.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fnd.c'end.
cl.d.
cl.g.
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PLATE 5.

Figs. 32–34. Amia calva, 8th day. Three successive sagittal sections of the anterior portion of the mesencephalon, showing the optic reflex cells and their axons uniting within the mesocoele to form the fibre of Reissner. Figure 32 is median. In these figures anterior is at the right, and dorsal is toward the upper left-hand corner of the plate. × 205.

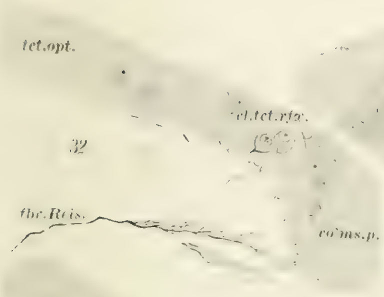
Fig. 35. Amia calva, 17th day. Sagittal section of the anterior portion of the mesencephalon, anterior at left. The system of axons within the mesocoele is somewhat more consolidated. × 205.

Fig. 36. Amia calva, 5th day. Transverse section through the roof of the mesencephalon, caudad of the posterior commissure, showing the optic reflex cells. × 100.

Fig. 37. Amia calva, 5th day. Transverse section through the roof of the mesencephalon, and edge of the posterior commissure flexure, showing the recessus of mesocoele above the posterior commissure. × 87.

Fig. 38. Amia calva, 8th day. Transverse section through anterior portion of the mesencephalon and posterior commissure, anterior to preceding. × 120.

Fig. 39. Amia calva, 4th day. Sagittal section through the posterior portion of the cord, showing a single posterior canal-cell and its axon. × 1200.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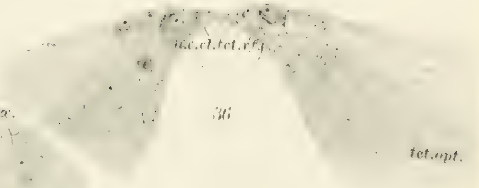
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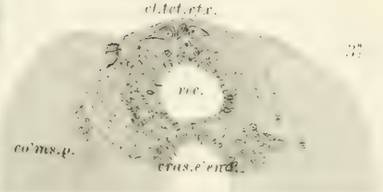
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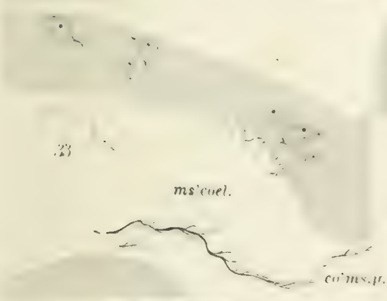
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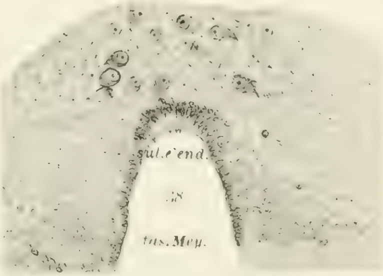
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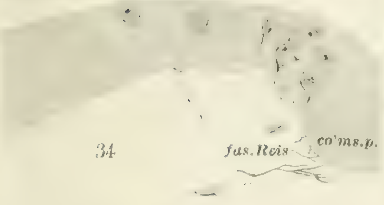
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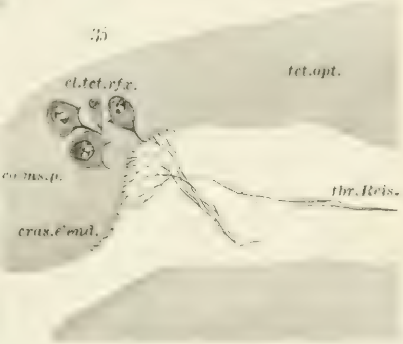
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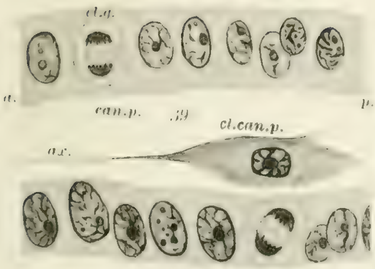
39
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a.
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PLATE 6.

Fig. 40. Amia calva, adult. Sagittal section, anterior at right, through the anterior portion of the mesencephalic roof and posterior commissure, showing the axons of the optic reflex cells uniting in the ventricle. Combined from several adjacent sections. × 41.

Fig. 41. Polypterus bichir, adult. Parasagittal section, anterior at right, through the anterior mesencephalic roof and posterior commissure, showing the relation of the optic reflex cells to the posterior commissure, and the axons uniting in the ventricle to form Reissner's fibre. Combined from several adjacent sections. × 41.

Fig. 42. Polypterus bichir, adult. Parasagittal section, anterior at right, of the anterior portion of the left optic lobe, some distance laterad of the preceding, showing some of the laterally lying optic reflex cells. × 87.

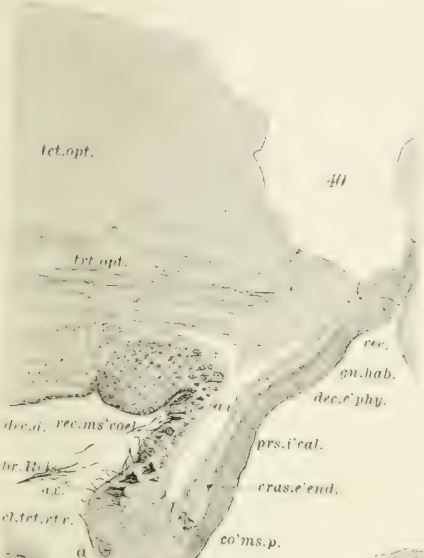
Fig. 43. Ameiurus nebulosus, larva, 15 cm. Sagittal section of posterior end of the spinal cord, showing Reissner's fibre and two posterior canal-cells. × 900.

Fig. 44. Lepidosteus osseus, larva, 1st day after hatching, 10 mm. Sagittal section anterior at left, of mesencephalic and diencephalic roof, showing the optic reflex cells whose fine axons project into the ventricle. The posterior commissure is not yet developed. × 205.

Fig. 45. Lepidosteus osseus, larva, 15 mm. Parasagittal section (tangential), anterior at right, of posterior portion of spinal cord, in part through the central canal, showing the branches of Reissner's fibre entering the cord. × 380 ca.

Fig. 46. Lepidosteus osseus, larva, 25 mm. Sagittal section, anterior at left, of the posterior end of spinal cord and ventriculus terminalis, showing two posterior canal-cells. × 470.

40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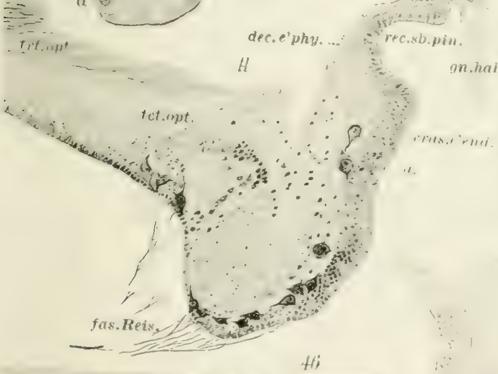
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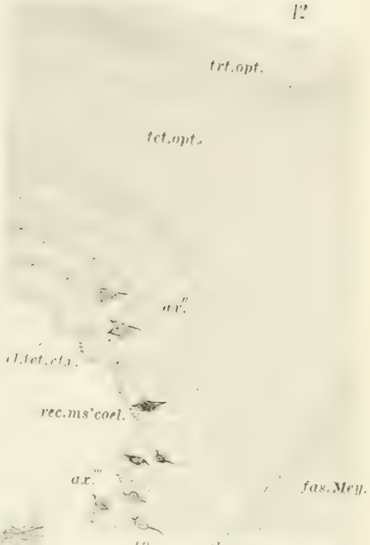
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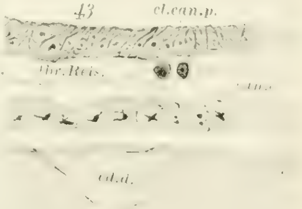
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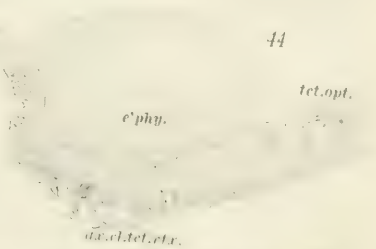
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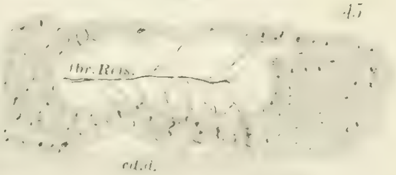
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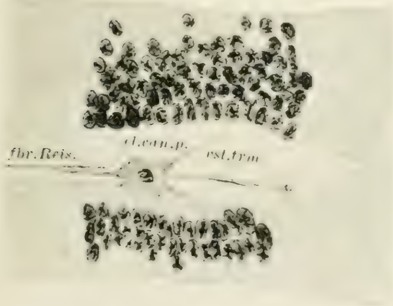
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PLATE 7.

Figs. 47–52 are parasagittal sections, anterior at the left, of the anterior roof of mesencephalon, and torus longitudinalis, cutting the posterior commissure, and showing something of the mode of formation of Reissner's fibre in the ventricle. Some details of the branches of the fibre have been drawn in from adjacent sections.

Fig. 47. Ameiurus nebulosus, young, 4 cm. long. The midbrain roof is of the primitive ganoid type. (Compare Figs. 25, 26, Plate 4.) × 205.

Fig. 48. Notropis cornutus, adult. Fascicles of Reissner's-fibre axons enter the mesocoele from the posterior side of the posterior commissure. × 87.

Fig. 49. Alutera schoepfii, young, 15 cm. long. Reissner's fibre is formed almost exclusively from fascicles of axons entering the ventricle anterior to the posterior commissure. × ca. 100.

Fig. 50. Rhombus triacanthus, young, 4 cm. long. Both the anterior and posterior branches of Reissner's fibre are shown. × 120.

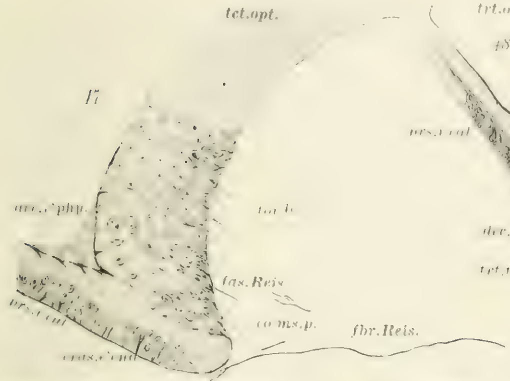
Fig. 51. Salvelinus fontinalis. The section is somewhat oblique; the anterior portion of the figure is median; the posterior portion parasagittal. Note the anterior branches of Reissner's fibre entering the ventricle anterior and ventral to the posterior commissure, and the posterior branches in the recess above the commissure. × 120

Fig. 52. Gadus callarias, larvae, 15 mm. long. Reissner's fibre ventral to the posterior commissure. × 385.

Fig. 53. Cynoscion regalis, adult. Transverse section of posterior commissure and Reissner's fibre. × 900.

Fig. 54. Another section of the same, showing more clearly the medullary sheath, and the punctate appearance in transverse section of the axillary portion, due to the cut ends of the constituent axons of Reissner's fibre. × 1350.

47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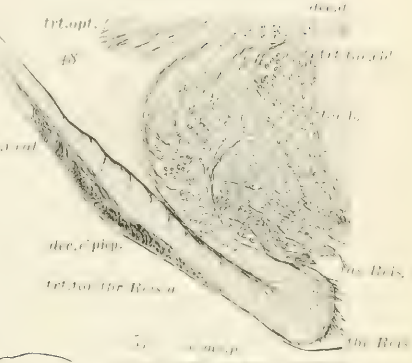
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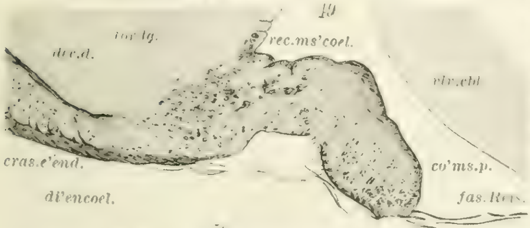
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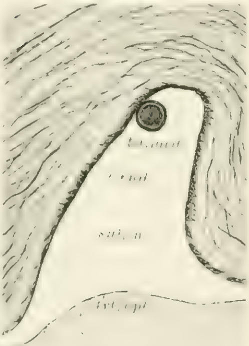
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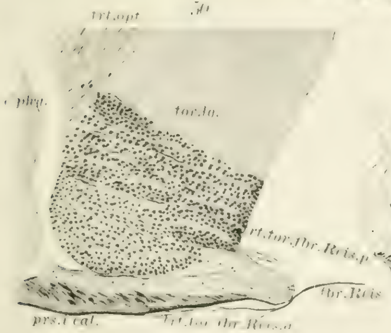
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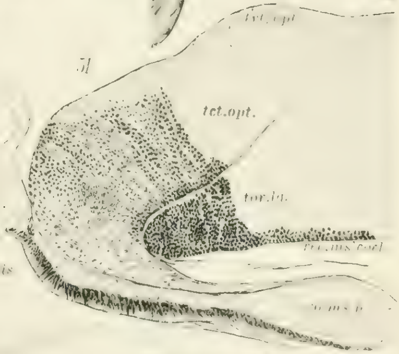
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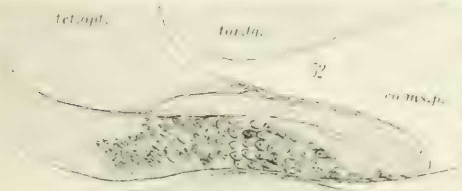
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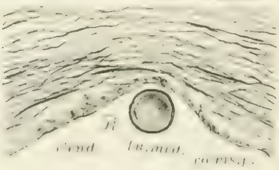
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PLATE 8.

Fig. 55. Tautogolabrus adspersus, larva, 2 cm. long. Parasagittal section through the anterior portion of the torus and posterior commissure, somewhat oblique, the lower portion of the figure being more nearly median than the upper. × 205.

Fig. 56. Tautogolabrus adspersus, larva, 25 mm. long. Sagittal section of the anterior portion of the torus and posterior commissure. × 205.

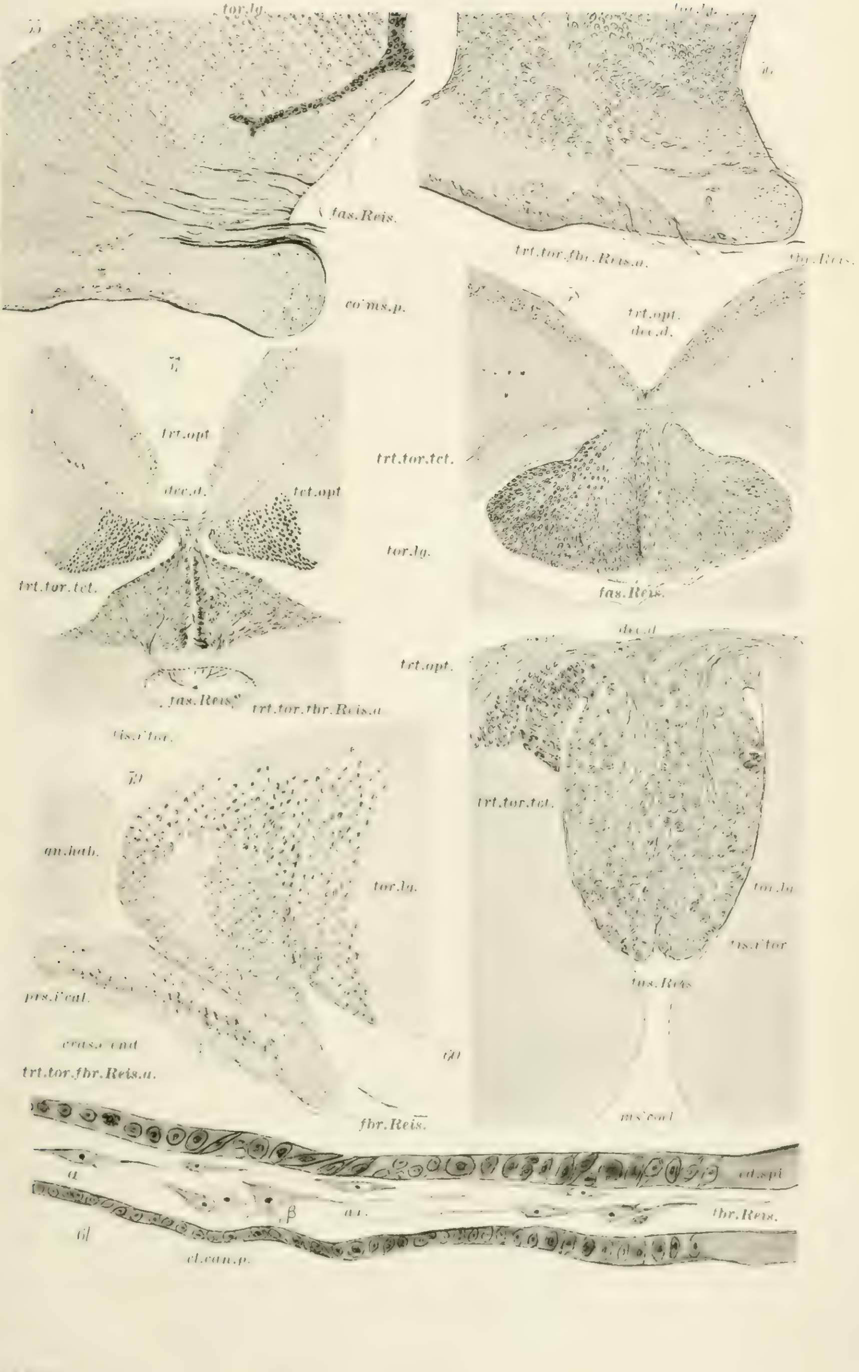
Fig. 57. Tautogolabrus adspersus, young, 15 cm. long. Transverse section through the torus and posterior commissure, the details filled in from three adjacent sections. × 87.

Fig. 58. Menidia notata, adult. Transverse section of the torus longitudinalis and adjacent portions of the tectum opticum, caudad of the posterior commissure. × 87.

Fig. 59. Fundulus heteroclitus, larva, 10 mm. long. Sagittal section of the anterior portion of the midbrain roof. × 205.

Fig. 60. Salvelinus fontinalis, larva, 25 mm. long. Transverse section of the torus longitudinalis and mesal portion of the tectum opticum, just caudad of the posterior commissure. × 205.

Fig. 61. Ameiurus nebulosus, 15 mm. long. Sagittal section of the posterior portion of the cord. The posterior canal-cells are drawn in from two adjacent sections. × 1000.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a.1.
fbr.Reis.
61
cl.can.p.

PLATE 9.

Fig. 62. Paralichthys dentatus, adult. Transverse section of the mesencephalon and torus longitudinalis through the posterior commissure. × 15.

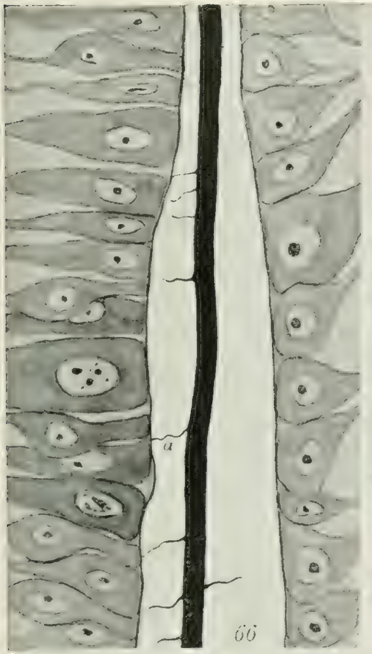
Fig. 63 Cynoscion regalis, adult, section as above. × 15.

Fig. 64. Amblyopsis spelaeus, adult. Transverse section of the roof of the mesencephalon and torus longitudinalis, caudad of the posterior commissure. × 40.

Fig. 65. Ameiurus nebulosus, adult, section as above. × 40.

Fig. 66. Pseudopleuronectes americana, adult. Sagittal section through the canalis centralis, in the middle portion of the cord, showing the branching of Reissner's fibre. Anterior is uppermost and ventral at the left. × 1000.

Fig. 67. Squalus acanthias, embryo, 10 mm. long. Sagittal section through the posterior portion of the canalis centralis, showing two of the posterior canal-cells, and some of the fine branches entering the ventral wall of the canal. × 1350.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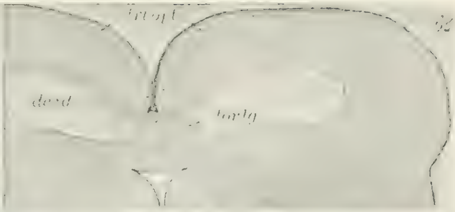
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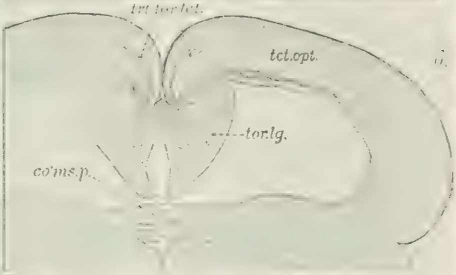
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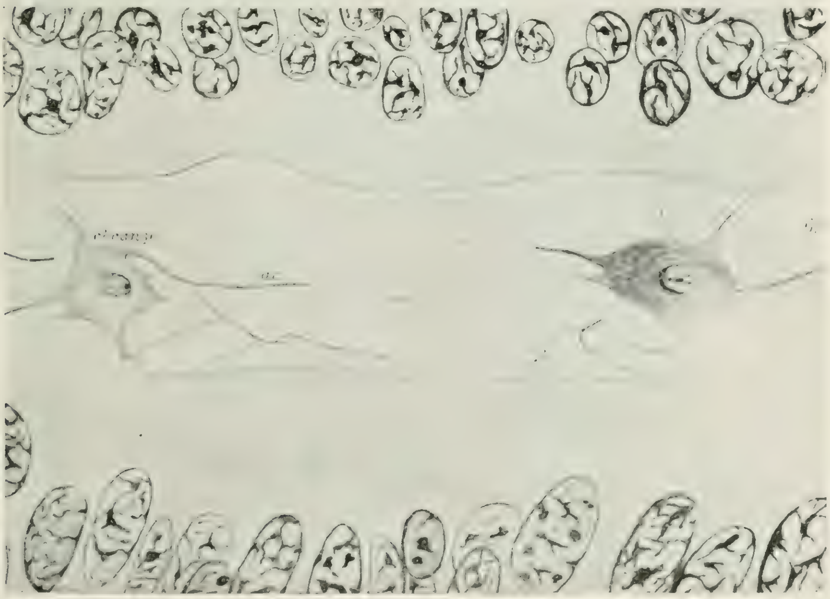
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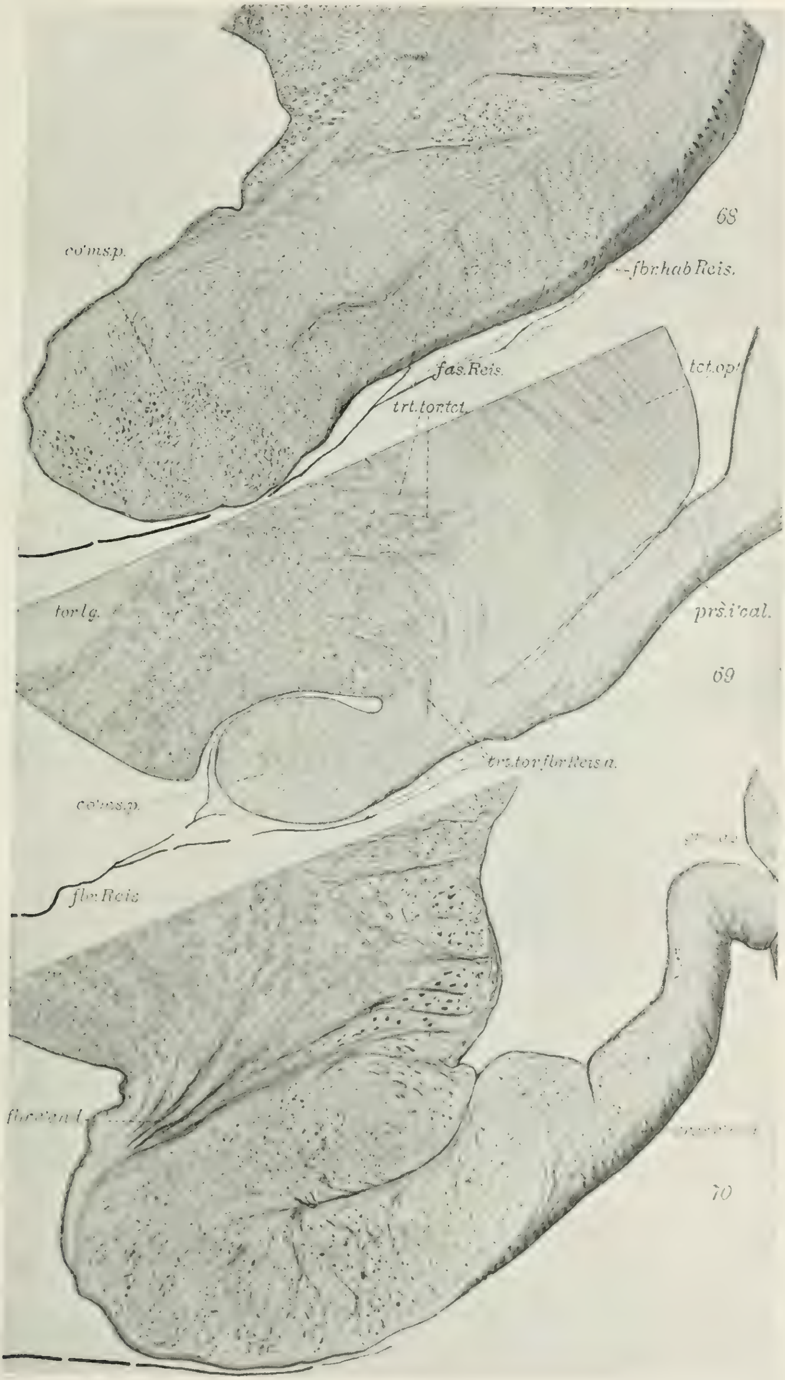
PLATE 10.

In all the figures the anterior end is at the right, and the dorsal side is toward the upper left-hand corner of the plate.

Fig. 68. Morone americana, adult. Sagittal section through the posterior commissure and adjacent region. × 120.

Fig. 69. Gasterosteus bispinosus, adult. Parasagittal section of the anterior portion of the midbrain roof. × 87.

Fig. 70. Microgadus tomcod, adult. Parasagittal section through the posterior commissure region. × 100.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co'ms.p.
fbr.Reis.
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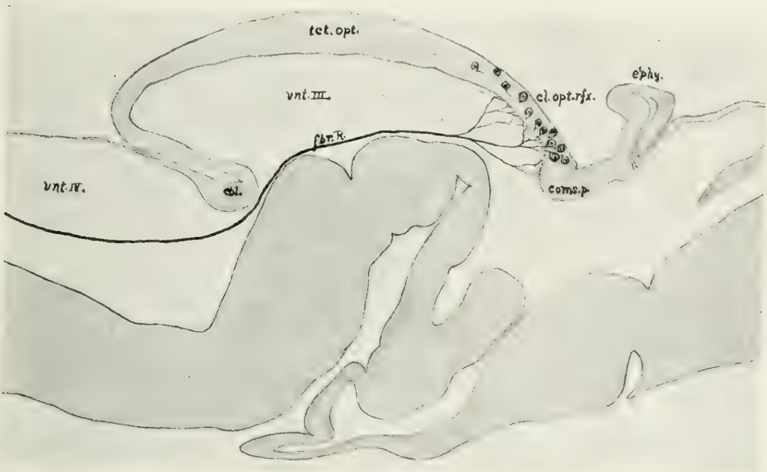
70

P.E.S. del.

PLATE 11.

Fig. 71. Raja erinacea, 14 cm. long. Diagrammatic sagittal section of the midbrain, showing the 'Dachkern' and the fibre-tracts arising from it, and the method of formation of Reissner's fibre.

Fig. 72. Amia calva, advanced larval stage. Sagittal section of the midbrain, somewhat diagrammatic, showing the mode of formation of Reissner's fibre.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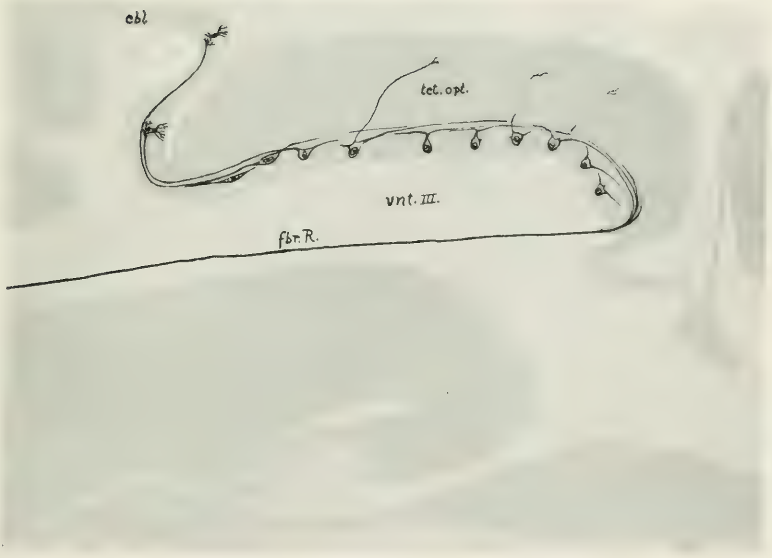
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THE MATURATION, FERTILIZATION, AND EARLY CLEAVAGE OF HAMINEA SOLITARIA (SAY).

By W. M. Smallwood.

With Thirteen Plates.

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I. Introduction.

During the summer of 1897, while at Wood's Hole, Dr. E. G. Conklin suggested that I work up the natural history and development of Haminea solitaria. The work has been carried on at Syracuse University, at Wood's Hole, for three seasons, and, as opportunity offered, in connection with my work at Allegheny College. The study of maturation and fertilization has been completed during the past year at the Zoölogical Laboratory of Harvard University.

I am under especial obligation to Dr. E. L. Mark for many helpful criticisms. I have also received valuable suggestions from Drs. Conklin, Hargitt, Lillie, and others.

My first plan was to make a detailed study of cell lineage; but Haminea showed so little variation from Crepidula, Umbrella, Planorbis, and other Mollusca that it did not seem desirable to give to this problem the time necessary for its completion. The present paper is confined to the cytological changes that take place during maturation, fertilization, and the early cleavages.

Notwithstanding the great volume of cytological literature, there are still a number of unsettled questions relating to the early stages of development. Experimental study has thrown doubt on some accepted conclusions and suggested new questions. Before we can adopt the new conclusions, we should find some confirmation of them from a study of development under normal conditions. The question of the individuality of the chromosomes, the changes and the permanency of the centrosome, the precise nature of fertilization, — these and other problems are still unsettled.

II. Collection of Material and Technique.

The opisthobranch Haminea solitaria is common along the Atlantic coast from Cape Cod to Florida. The species that occurs at Wood's Hole is undoubtedly identical with those that have been described from various other places along the coast, the differences in description being due chiefly to the age of the animals examined. Haminea solitaria is not commonly seen except in the breeding season, which at Wood's Hole extends from June to September. During this time the animals are found in favorable places in large numbers. The rest of the year they live in deep water.

Sometime, usually between the middle of June and the middle of July, Haminea migrates from the deep water into shallow ponds and lagoons, where the eggs are deposited on the mud, eel-grass, stones, sticks, etc. The development takes place so rapidly that to secure eggs in early stages of development, it was found necessary to collect the animals before the eggs had been laid. During the breeding season Haminea is not very sensitive to artificial conditions, so that it was possible to keep the animals under direct observation, although they died soon after egg-laying. During the summer of 1900, I was fortunate in collecting over forty adult individuals before copulation took place. Thus having the animals under direct observation and separated from all other forms, and knowing when copulation took place, I was able to secure eggs about the identity of which there could be no question. By acquiring a large amount of material, I have been able to work out a fairly complete series of the changes occurring in this period of development.

Under natural conditions Haminea solitaria deposits eggs at any time of day or night; but many more were laid between three and six o'clock in the morning than during all the rest of the twenty-four hours. In confinement there did not seem to be any time that was especially favorable for egg-laying. Eggs were usually laid from eight to twelve hours after copulation. My plan was to observe when copulation took place, then to isolate the individual that was about to lay, and watch until the process began. In this way I was able to secure a large number of eggs in the early maturation stages. By killing a number of animals during the process of deposition, a large amount of material was secured for future study.

It is practically impossible to determine the exact number of eggs in a single capsule, the size of the capsules varying much, and the number of the eggs being large. I should estimate that there were on the average about two thousand eggs in a capsule. An animal can produce a single capsule in forty-five minutes. When the capsule is finished, the eggs first deposited are in the two-cell stage. One hour after the capsule has been laid, all of the eggs are in the two- or four-cell stages, the development being much more rapid than is usual in Mollusca.

In general, the technique involved has been very simple. The ordinary fixing reagents were tried. Kleinenberg's picro-sulphuric and Conklin's picro-acetic mixtures gave the best results; the latter, being much the more satisfactory, was exclusively used on the material described in this paper. A whole capsule, when dropped into the fixing fluid, even if left a long time in it, often failed to be penetrated. To obviate this difficulty, the capsules, after they were placed in the fixing fluid, were cut up into small pieces with wooden needles, which could be used without much danger of crushing the eggs. The eggs were left in the fixing fluid for one hour, then placed in 70% alcohol, which was frequently changed, where they remained until the color due to picric acid was removed. The removal of the water from the albumen surrounding the eggs causes the whole mass to stick together. This has a certain advantage, for it allows the later treatment of the eggs in one mass, instead of singly. There is a strong tendency for particles of dirt to adhere to the outside of the capsule; this often renders cutting difficult by increasing the liability of the sections to tear.

For staining, use was made of Heidenhain's iron-hæmatoxylin, followed by an aqueous solution of Bordeaux red as a plasma stain. This method gave excellent results for all except fertilization stages. In order to differentiate the sperm within the egg from the deutoplasm, the eggs were stained in Delafield's hæmatoxylin and differentiated with a weak solution of picric acid in 90% alcohol. The picric acid renders the deutoplasm reddish yellow, but leaves the sperm black. This is also a good stain for the maturation stages and has been used as a control of the iron-hæmatoxylin method.

During the current year a number of the preparations have been restained with Brazilin (Hickson, :**01**), which has given results even better than the iron-hæmatoxylin. The eggs after being sectioned and mounted were mordanted in a solution of iron in 70% alcohol for periods varying from thirty to sixty minutes, and then stained from thirty minutes to two hours in a ½% solution of pure Brazilin in 70% alcohol. The Brazilin gives a double stain: the centrosomes, chromosomes, and microsomes are stained intensely black, while the cytoplasm takes a color resembling that of a Bordeaux-red stain; but the cytoplasm is not often overstained, as frequently occurs with Bordeaux red. This stain has the further advantage over the iron-hæmatoxylin that the process is shorter and the tissues do not have to pass from alcohol to water. It also rarely overstains; and even when it does the excess may be removed by simply washing the preparation in 70% alcohol, or dipping it in the mordant for a few seconds.

III. Observations.

1. Growth of the Egg.

The eggs of this species when full-grown lie free in the follicles of the ovotestis. A follicle may contain eggs only, spermatozoa only, or both eggs and spermatozoa. It is impossible to show that any part of the ovotestis is exclusively concerned in the production of either of the sexual elements.

If an animal is examined after egg-laying, there are rarely found any full-grown eggs, or even eggs partly filled with deutoplasm. This is in striking contrast to what is found in Limax (Byrnes, '**99**), where relatively few eggs mature simultaneously.

For two reasons no attempt has been made to work out the ovogenesis or spermatogenesis: first, because the early changes take place when the cells are very small; secondly, these changes take place in the spring, while the animals are living in deep water, where they would be inaccessible except by dredging, even after their habitat had been discovered.

The cytoplasm of a young ovocyte presents a granular appearance which is perfectly uniform in all regions of the cell (Plate 1, Fig. 2). I have tried several plasma stains, but have been unable to demonstrate a yolk nucleus, such as Crampton ('99) finds in Ascidians, and others have found in other animals; nor do I find that the surrounding cells are taken into the cytoplasm as in Helix (Obst, '99). The egg simply increases in size, this increase being accompanied by the appearance of deutoplasmic bodies in the cytoplasm, which at first are few and small. After these yolk spheres have begun to be formed, they increase rapidly in number, until there is no evidence of any substance outside the nucleus and inside the egg membrane except deutoplasmic bodies.

The changes which take place in the nucleus can be easily observed. In the young ovocyte the nucleolus is a solid mass staining uniformly (Fig. 2). From it there often radiate more or less regularly arranged linin fibres, which give to the nucleus a radiate appearance. As soon as the deutoplasm begins to appear in the cytoplasm there can be distinguished in the nucleolus clear regions (Fig. 3), at first very minute, afterwards larger and usually round; these vacuoles increase in size and number, adjacent ones fusing into larger vacuoles, and these eventually uniting into one large cavity, such as are shown by Montgomery ('98) for Piscicola rapax and Tetrastemma elegans. During the growth of the egg, the nucleus increases in size proportionately to that of the whole egg. Owing to the increase in the size of the nucleus, it is difficult to say whether in the early stages the stainable material of the nucleolus is disappearing, or whether the nucleolus is simply enlarging and the added space is being occupied by the fluid in the vacuoles (Plate 1, Fig. 3; Plate 2, Fig. 7). However that may be, as the egg begins to reach full size, the nucleolus becomes filled with a non-staining fluid, the dark staining portion diminishing and finally disappearing. It has not been possible to demonstrate any structure in the vacuoles of the nucleolus; this agrees with the observations of Korschelt ('95) on Ophryotrocha and Montgomery ('99) on Piscicola and Tetrastemma, but differs from those on Tubifex rivulorum and Clepsine complanata by Gathy (:00), who finds a definite network in the nucleolus at a corresponding stage. Coe ('99, p. 436) finds in Cerebratulus also a network in the vacuole. The vacuoles in the nucleolus of Ophryotrocha, as observed by Korschelt ('95, pp. 562–573), are in the early stages similar to those in Haminea, but in Ophryotrocha they do not fuse into one large structure occupying the whole of the nucleolus.

As compared with the young ovocyte, the full-grown egg (Plate 1, Fig. 1) has increased very much in size and is altered in appearance. The deutoplasm completely fills the region which in the young ovocyte was occupied by the granular cytoplasm. It is impossible to note the structure of the cytoplasm at this stage because it is so scanty and so crowded in between the yolk spheres. The nucleus is large, usually roundish, and centrally located. It is limited by a definite membrane, which may be irregular in outline in poorly fixed material.

The nucleolus is a thin-walled vesicle filled with a non-staining fluid. The chromatin has entirely disappeared, or is located in the form of a crescent on one side of the nucleolus. This increase of the non-staining portion of the nucleolus is brought about by the gradual confluence of the vacuoles, which at first usually arise on one side of the nucleolus (Plate **2**, Figs. 7, 8); the deeply staining chromatin diminishes in proportion to the non-staining material. In a number of cases observed, a clear region within the nucleolus enclosed a deeply staining mass, which was the remnant of the chromatin of the nucleolus. Coe ('**99**, Fig. 13) shows a similar condition in the ovum of Cerebratulus.

The nucleolus is filled with a network of fibres that are loosely interlaced. These have on them deeply staining masses, which are especially noticeable where two fibres cross. This condition is prominent in eggs that have been stained with iron-hæmatoxylin. There may or may not be present one or two plasmosomes. When a strong plasma stain is used, the appearance of the nucleus is entirely changed (Plate **2**, Fig. 8). The fibres are distinguished with difficulty, and the region seems to be occupied by fine granules uniformly distributed. Heidenhain (**'93, '94**) terms these two substances, which are thus differentiated by a distinctively nuclear and a distinctively plasma stain, basichromatin and oxychromatin, respectively. This distinction is very evident in Haminea, the full-grown nucleus presenting different appearances according to the method of staining employed.

In these changes I have been unable to detect any evidence of a centrosome. Nor is there any distinguishable polarity to the egg as it lies in the follicles. The nucleus is in the centre, and does not take up an eccentric position during these changes, nor even at the beginning of maturation.

2. MATURATION CHANGES.

(a) *Before Deposition.*

Centrosome. Notwithstanding the fact that a large number of persons have studied the mitotic phenomena in Mollusca, all, with the exception of Conklin (:01),[1] have failed to trace back the centrosome to its earliest condition. In most cases recorded, such as Byrnes ('99), Linville (:00), Lillie (:01), and others, the earliest centrosomes found have been well formed, have lain on opposite sides of the germinative vesicle, and have been connected to each other by a well-formed central spindle, the chromosomes of which lay in the equatorial plane, — the beginning of the metaphase.

In Haminea a distinction can be made between the centrosome (inclusive of the centriole) and the differentiated cytoplasm immediately enveloping it. To the latter I shall apply the term *cortical layer* (*st. ctx.*); but it is to be noted that this cortical layer becomes differentiated into two zones, — a medullary zone (*st. med.*) and a peripheral or cortical zone (*st. ctx.*). These conditions will be discussed in the section "After Deposition" (pp. 279, 280 ff.).

The eggs of Haminea pass through several maturation changes while still in the ovotestis. I have made a number of attempts to secure eggs at the beginning of mitosis; but in every animal examined after egg-laying had begun, all of the ovarian eggs, even if they still remained in the follicles, had a partly formed spindle extending through the germinative vesicle with a definite centrosome at each pole. This made the getting of early stages of maturation difficult. It is mere chance if one kills an animal when the ova are at the beginning of their metamorphosis, for there is no external sign to indicate this epoch, such as there is after the beginning of copulation. The advanced condition following copulation is very regrettable. When we consider that the living animals are not common, and that it is necessary after collecting them and before killing them, first, to observe when they copulate, and afterwards to watch until they show signs of laying, the difficulty of the problem becomes evident. However, stages have been secured that are much earlier than those usually observed in Mollusca. They must be regarded, I think, as representing a very early phase of karyokinesis, for the centrosome (*c'so.*) in the earliest stage observed is not a homogeneous single body; it consists of at least five spherical masses placed in close apposition (Plate 1, Fig. 4). The clear area that appears in the centre of this group of bodies is due to their arrangement. This is shown by focusing, there being no sharp or definite limit to the area. The outline of the centrosome does not become regular until the chromosomes reach the metaphase. The general appearance of this early centrosome in Haminea is similar to that shown by Conklin (:01, Diagram A, B) for Crepidula; he, however, shows only four bodies surrounding a clear area. But the further history of these bodies in Haminea does not agree with the changes that take place in the corresponding stages in Crepidula. By following the successive stages shown in Figures 4–6 and 9–13 (Plates 1–3), it will be seen that the spherical masses composing the centrosome become smaller and more regular in outline; the clear area diminishes in size until it disappears, and the five bodies meantime become a small, deeply staining, homogeneous centrosome. These changes result in the formation of a body which in its reaction to stains, its density, and its further development shows that it is probably homogeneous. While it is possible that this body has been formed by the differentiation of one of these five bodies from the others, it is more probable that it has resulted from a fusion and condensation of all of the bodies into one.

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[1] Had Professor Conklin's most admirable monograph on "Karyokinesis and Cytokinesis in the Maturation, Fertilization and Cleavage of Crepidula and other Gasteropoda" (Jour. Acad. Nat. Sci. Philad. XII. 1902) appeared before this article was sent to press, I should have attempted to include an extended comparison of Crepidula and Haminea.

A polar view of the centrosome (Plate 2, Fig. 5) shows these bodies at their maximum size; whereas an oblique section (Fig. 6) usually shows only part of the bodies. Their reaction to stain is another noticeable characteristic. In Figure 4, the earliest stage observed, they take a very dark stain in iron-hæmatoxylin. In the next two stages (Figs. 9, 10) the stain is not as dark; this may be due in part to the smaller size of the bodies, but it seems as if the bodies were changing chemically and therefore reacted differently as they passed through these changes.

It is difficult to decide with certainty how these bodies become converted into a small, homogeneous granule, like a typical centrosome. In sections of the ovotestis of animals that have just begun to lay, one often finds in a single section from fifty to one hundred eggs in approximately the same stage of development; so the difficulty has not been so much want of material as inability to interpret satisfactorily the conditions presented. An attempt was made to ascertain whether any of these bodies pass off *in toto* into the cytoplasm, but there is no satisfactory evidence of this. Owing to the large number of stages studied, it was possible to draw an inference as to what was taking place. It seemed evident that these bodies were becoming less clearly defined, as well as smaller in size. There is no evidence, however, that they break up and then pass into the cytoplasm; nor is there anything to show that in Haminea (as in Unio, Lillie, :01) one of these bodies becomes centrally located and alone constitutes the centrosome of the second maturation spindle. Lillie (:01), it will be recalled, found in Unio a similar mass of granules at the beginning of the formation of the second maturation spindle; and one of these bodies became the centrosome, while the others formed a row of microsomes that marked the outer limit of the medullary zone, Lillie's "inner sphere," and finally passed into the cytoplasm as indistinguishable granules. Such is not the case in Haminea, — at least another explanation is possible: a gradual reduction in size and a fusion of the five bodies into one that becomes smaller than any of the five in the earliest observed stage. The question as to what becomes of the disappearing substance is partly explained by observing the changes that take place in the stages noted. These stages form a connected series, as can be shown by noting the sequence based on the known changes in the chromatin, and on the formation of spindle fibres connected with the chromosomes. The reaction to stains does not seem to furnish sufficient evidence to decide in favor of either a physical or chemical explanation of the change. While it is barely possible that these bodies may break up and the parts become indistinguishable from the deeply staining cytoplasm surrounding them, it seems more probable to me that they change chemically, and then gradually become united into one body.

While the egg is still in the ovotestis, the centrosome begins to be differentiated into two concentric regions. These can be seen in Figure 16 (Plate 3), a very narrow zone enveloping the deeply staining central part. This outer region takes such a deep plasma stain that it is difficult to distinguish the two. A further description of these parts will be taken up in the next section.

Cortical layer. The centrosome is surrounded by a finely granular mass, which shows neither yolk spheres nor the reticulate structure of the cytoplasm which occupies a still more peripheral position (Plates 1–3, Figs. 4–6 and 9–13). This mass takes a deeper stain than the surrounding cytoplasm, and has a decided affinity for plasma stains, especially in the earlier stages. Van Beneden et Neyt ('87) named this the "couche corticale." Conklin (:01, p. 287) says, "This substance is not self-propagating, but arises anew in each cell generation, being composed of nuclear sap and of dissolved oxychromatin from the nucleus, and of hyaloplasm from the cell body." The cortical layer nearly envelops the germinative vesicle, as can be seen in favorable preparations by focusing. In some stages I have found a thin layer of it (Plate 2, Fig. 11) extending entirely around the germinative vesicle. This is still more evident in a cross-section of the equatorial region of the spindle (Plate

3, Figs. 14, 15, 17). In the early stages this cortical layer is well defined, but as the egg develops it becomes less marked, until there is no distinct limit between it and the cytoplasm.

Astral rays. The astral rays are best studied when stained with hæmatoxylin and differentiated with dilute picric acid. When this method is used, the primary rays can be traced from the region of the poles to the periphery of the egg. The rays end peripherally in a branched meshwork that is continuous with the common reticulum of the cytoplasm (Plate **3**, Fig. 18). These primary rays are easily seen and are arranged quite regularly, like the spokes of a wheel; along each side of them are arranged a large numbei of minute bodies (microsomes ?). These bodies are not found on the rays in the region immediately outside the cortical layer. They are more abundant in the earlier stages than at any subsequent time. In between the primary rays are found some of the deutoplasmic spheres and the coarser structures of the cytoplasm. The secondary rays (Plate **3**, Fig. 18) are very much shorter and more numerous. They do not extend far beyond the cortical zone, and do not bear any evidence of being accompanied by such bodies as occur along the primary rays.

I have been unable to determine in Haminea how the rays are formed in these early stages, but in the formation of the second polar spindle the rays extend out from the region of the centrosome, as in Toxopneustes (Wilson, **:01**^{a}). According to Wilson, the rays are paths along which hyaloplasm is moving toward the centre of the aster. Other views have been advanced in regard to the character and origin of the rays. Hill (**'95**) states that they are formed from a central point and grow outward. Child (**'98**, p. 394) considers them to be the expression of an activity; he holds that they come from the cytoplasm, and are mere temporary structures. Mead (**'98**) argues that the rays are a part of the general reticulum of the cytoplasm. Van Name (**'99**) states that in the case of the first polar spindle the rays from the centrosome at first seem to have little connection with the cytoplasmic reticulum, but that later they branch and grade into the reticulum. Gathy (**:00**, p. 55) says, in the case of Tubifex, "Les rayons de l'aster sont formés par des cordons de protoplasme qui s'orientent radialement." Lillie (**:01**, p. 231) thinks that the fibres may develop from a common reticulum or foam-structure.

This is a question that can be satisfactorily settled only upon eggs which permit of being studied in the living as well as in the fixed condition. It would seem from Wilson's (**:01**^{a}) account that the rays must be regarded as thepaths along which particles move toward the centre of the aster. It is certainly very evident in the later stages of Haminea that the rays lengthen out toward the periphery, being at first short, and that their length is correlated with the size of the spindle; that is, that they increase in length up to the metaphase.

Chromosomes. Between the adult ovarian egg and the earliest maturation stage discovered, profound morphological changes take place in the chromatic matter, — changes which do not, however, occupy any considerable time; for, as has been stated, an animal lays a whole capsule in the course of forty-five minutes, the eggs always being in the metaphase when laid. I have made repeated efforts to bridge over this gap in earlier maturation, but without success. When one studies the germinative vesicle of this stage (Plate 1, Fig. 4), he is impressed by the fact that it is a very early stage in the formation of the achromatic figure. The paucity of chromatic matter is at once evident. If any spireme has been formed, it does not follow the usual course, nor does it appear to give origin to chromosomes. In this early stage the germinative vesicle, already much elongated, is filled with a loosely branched linin network, which often has at its nodes noticeable chromatin masses. There is to be seen in connection with the linin one or more irregular vesicles, each containing one or two chromatic masses. Because of the further changes that these undergo, I have termed them chromosome vesicles (*vsl. chr'so.*). But they are different in origin and function from structures in the spermatogenesis of Brachystola magna similarly named by Sutton (:00). It is difficult to be certain just how these vesicles arise; ultimately sixteen such vesicles are formed. I have been able to count thirteen vesicles in one favorable section. At first these chromosomal vesicles are more or less irregular in outline, but they soon assume an elliptical form (Plate 2, Figs. 9–12). As the outline becomes more definite and regular, there appears, on the inside of each, chromatin matter, which is usually distributed along the inner surface of the wall of the vesicle (Fig. 9). Soon the chromatin begins to be concentrated, at first on one side of the vesicle usually; it then extends out toward the centre of the vesicle (Figs. 11–12). This chromatin mass is broadly connected with one side of the vesicle, and a few delicate chromatin threads, or sometimes only one, extend from it across the vesicle in various directions. These chromatin threads are best seen in cross-sections of the spindle (Plate 3, Fig. 17). They disappear as the chromatin increases in amount and definiteness of form. The masses within the vesicles now begin to assume a form which is very characteristic for Haminea, and we may speak of them from this time on as the chromosomes. Each chromosome is more or less distinctly trilobed, as though constricted, having the form of a linear series of three beads (Fig. 13). In the typical condition, each of the three parts is of equal size, and all are arranged in a straight row. It often happens, however, that the three masses are not arranged in a straight line, the result being various odd shapes. The elbow-like form (Plate **3**, Figs. 14, 16), so common in Unio, arises in Haminea by a lateral displacement of one of the end masses. In one instance the chromatin was found in the early stage in a typical tetrad condition, each of the four masses being held together by linin (Plate **2**, Fig. 9).

The chromosome vesicles contain in addition to chromatin and linin a substance, probably a fluid, which takes a faint plasma stain. This substance is more abundant in the peripheral region of the vesicle, which in section gives to the wall of the vesicle an irregular outline on its inner surface (Plate **2**, Figs. 9, 11, 12; Plate **3**, Figs. 15, 17). As the chromatic matter increases in amount, the substance taking a plasma stain diminishes until there is no staining reaction in it.

A broad band of fibres extends from each vesicle to the centrosome. In the earlier stages there are granules on the fibre (Plate **2**, Fig. 9). When the vesicles lie in the centre of the germinative vesicle, the arrangement of the fibres gives the appearance of a central spindle.

With the concentration of the chromatin into a single definite mass, the walls of the chromosome vesicles become less distinct and soon disappear, simply fading away, probably being dissolved *in situ*. As soon as the walls have disappeared, the chromosomes lie free at the equatorial plane of the spindle (Plate **3**, Fig. 16). The maturation figure now has the typical appearance, except that the chromosomes still retain their tripartite condition.

There are three possible ways in which to account for the formation of the chromosomal vesicles. It is possible that the bodies which I have termed plasmosomes (p. 266) may be karyosomes, and that each of them may have given rise to a vesicle; but this view loses its force when we remember that these plasmosomes may or may not be present in the ovarian egg, and that the other vesicles are formed independently of the first one.

It is more probable that we are to look for the origin of the vesicles in a metamorphosis of the linin itself; in which event there are two possible explanations. First, there may be a direct rearrangement of the linin to form a vesicle. This is probably true in part, but does not seem fully to explain what happens. Secondly, the chromatin masses located at the nodes may by vacuolation gradually form quite a regular vesicle.

The chromatin masses are held together by the linin. In Figure 4 we can see various places in the linin, where, if we imagine the network to be the optical section of alveoli, we really have a series of vesicles. That these are closed vesicles can be made probable by a comparison of longitudinal and transverse sections, since in each case the limit is an unbroken wall.

At the same time that the linin disappears, these chromosome vesicles increase in number. This would seem to indicate that there is a genetic connection between the two. When the vesicles (*vsl. chr'so.*) are viewed in cross-section (Plates 3, 4, Figs. 14–17), one sees that they are literally held in place by the linin, the linin being directly continuous with the walls of the vesicle. This is evident only in the earlier stages.

It is at once evident that Haminea exhibits an unusual process in regard to the formation of the chromosomes. The ultimate result is the same as in other animals, but the intermediate steps are different from those presented by the usual spireme. The chromosomes do not split until the metaphase; this is a strong argument for the view that no regular spireme has been formed in the stages which precede the earliest conditions observed. Wilson (:00, pp. 69–70) in describing indirect division says: "The metaphase, which follows, forms the initial phase of actual division. Each chromosome splits lengthwise into two exactly similar halves, which afterwards diverge to opposite poles of the spindle, and here each group of daughter-chromosomes finally gives rise to a daughter-nucleus. In some cases the splitting of the chromosomes cannot be seen until they have grouped themselves in the equatorial plane of the spindle; and it is only in this case that the term 'metaphase' can be applied to the mitotic figure as a whole. In a large number of cases, however, the splitting may take place at an earlier period in the spireme stage, or even, in a few cases, in the reticulum of the mother-nucleus. Such variations do not, however, affect the essential fact that *the chromatic network is converted into a thread which, whether continuous or discontinuous, splits throughout its entire length into two exactly equivalent halves.*" In discussing the significance of the formation of the spireme, he adds (p. 245) the following: "Roux argued that the facts of mitosis are only explicable under the assumption that chromatin is not a uniform and homogeneous substance, but differs qualitatively in different regions of the nucleus; that the collection of the chromatin into a thread, and its accurate division into two halves, is meaningless unless the chromatin in different regions of the thread represents different *qualities* which are to be divided and distributed to the daughter-cells according to some definite law." The ultimate purpose of a spireme, then, according to this view, is lost unless its parts retain their identity; but it seems impossible to show that this is the case in Haminea. Wilson (:**01**^{a}, p. 572) finds that in Toxopneustes the chromosomes have different origins according as a strong or weak solution of magnesium chloride is used, "the chromosomes arising in one case from the nucleolus, in the other from the general chromatin reticulum." Similarly R. Hertwig ('**99**) found that in Actinosphærium the chromosomes had a different origin, depending upon whether the animal was well fed or starved. Wilson (:**01**^{a}, p. 575) concludes his discussion of the double origin of the chromosomes with the following statement: "It may be pointed out finally that the foregoing interpretation, if correct, seems to involve the further conclusion that chromatin, or at least the constituent on which the staining reaction depends, must be regarded as a liquid substance that may be absorbed or given off by an 'achromatic' basis such as plastin or linin, and thus may flow from one part of the nucleus to another. It seems to me further that the facts observed in the magnesium-eggs are not favorable to the hypothesis of the persistent individuality of the chromosomes." Delage (:**01**, p. 347) states that the number of the chromosomes "se rétablit par autorégulation: c'est une constante cellulaire spécifique, et la personnalité des chromosomes n'a rien de réel."

Van Beneden ('**83**) was one of the earliest investigators to suggest that the chromatin may give rise to the achromatic network and to the nuclear membrane. He further suggested that the chromatin might pass into a chromatic or a non-chromatic condition, according as it took up or gave off chromophilous substance. Coe ('**99**) finds in Cerebratulus that the nucleus loses its power of holding hæmatoxylin as soon as the egg is laid, the chromatin gradually reappearing as mitosis proceeds. Wilson (:**01**^{a}) has shown that the chromatin may pass through different stages, and that the basis of the chromatin must be regarded as a liquid. Hargitt (:**00**, :**04**) finds in Pennaria that after the nucleus breaks down during cleavage, the chromatin may entirely disappear, for at that time there is no chromatin reaction with any of the basic stains. Later, when cleavage is under way, the chromatin is again demonstrable.

In seeking to explain the source of the chromatin in Haminea, it is readily seen that the changes already described lend strong support to the views quoted. We have seen that there are to be distinguished two conditions of the chromatin, — the basichromatin and oxychromatin, —

and that in the germinative vesicle of Haminea, as the basichromatin decreases, the oxychromatin becomes more abundant. During the growth of the ovum until maturation begins, there is a gradual decrease in the amount of material which takes a basic stain. The first thing to form in the early maturation figure is a linin network. Upon this network there are gradually formed small masses of chromatin. The linin network and the masses of chromatin give rise to the chromosomes. Since, then, there is a stage where there is little or no visible chromatin, one must conclude that the chromatin passes through a non-staining condition, — probably, as Wilson maintains, a liquid condition, — and that it is re-collected in the chromosomal vesicles, where it again takes a basic stain.

The investigations in experimental cytology have yielded many new facts during the past few years, and the results are in many cases directly comparable with those following the normal activity of the egg. If these results are to be of permanent value and are to help in establishing general laws, they should be verified under conditions as far as possible normal. Haminea seems to me to afford confirmation of the results obtained by experimentation in regard to the liquid and nonstainable condition of the material which is to become the chromosomes. Here we have a perfectly normal process, taking place under natural conditions, which is in a measure parallel to the experimental results. The results, found under both normal and experimental conditions, make it probable that the persistent individuality of the chromosomes is not of universal occurrence; in Haminea the chromosomes do not have any connection with a spireme. This fact is interesting because of the significance that has been attached to spireme formation as noted in the passages quoted. We are hardly justified in drawing any radical conclusions from this one case, clear as it is. It does suggest, however, that the question is still open, and that one needs more observations before admitting as universal the occurrence of a spireme, and before accepting the supposed significance of its division as a definitely established universal fact.

(b) *After Deposition.*

(I.) First Maturation Figure.

In the interpretation and nomenclature of the centrosome in a former paper I (Smallwood, **:01**) followed Conklin's (**:01**) use of the terms of Van Beneden et Neyt (**'87**). Since that time has appeared the extended contribution of Boveri (:**01**) in which he discusses the centrosome and (p. 125) defines it as follows: "Ein Körper, an den die Sphärenradien direkt herantreten, ist das Centrosoma." The body which occurs at the centre of the "spheres attractives" is named by Van Beneden et Neyt ('**87**) the "corpuscule central;" it is this which, according to the definition of Boveri, is to be interpreted as the centrosome. The centrosome may become differentiated into two distinct regions, the centriole in the centre, and the centroplasm surrounding it. The latter is defined by Boveri (:**01**, p. 32) as follows: "Was nun die feinere Zusammensetzung dieses Centralkörperchens anlangt, so lässt sich in seiner Substanz, die ich fortan als Centroplasma bezeichnen will, bei keiner Untersuchungsweise eine Spur einer radiären Struktur erkennen." I have decided to use in my description of the centrosome Boveri's terms and definitions of centroplasm and centriole.

As long as the egg of Haminea remains in the ovotestis, the mitotic figure which replaces the germinative vesicle occupies a central position, and the deutoplasm constitutes a layer of practically uniform thickness at the periphery of the egg, the cytoplasm — free from yolk except for a few isolated spheres — being largely concentrated in the region immediately surrounding the mitotic figure. This condition is well illustrated in Figure 16 (Plate **3**). It is at first impossible to say which pole of the spindle will eventually reach the surface of the egg; but as soon as the egg is laid, the whole mitotic figure and the immediately surrounding cytoplasm move peripherally. The yolk thus comes to predominate in one portion of the egg, which we may now designate as the nutritive part. Figure 19 (Plate **4**) shows the mitotic figure migrating into a radial position. At this stage the yolk granules have disappeared between the outer pole of the spindle and the periphery of the egg. The cytoplasm accumulated here continues to occupy this region of the egg, which we may now designate as the formative part.

Centrosome. Figures 68 (Plate **10**) and 19 (Plate **4**) are from eggs fixed immediately after deposition. The centrosome, as compared with the conditions in an egg from the ovotestis, has increased in size; the centriole, though a single body, is irregular in outline; the centroplasm (Figure 21, *c'pl.*), the region immediately surrounding the centriole (*c'l.*), is distinct, and, like the centriole, slightly irregular in outline; it takes a very deep plasma stain, which in the early stages often makes difficult the task of distinguishing it from the centriole, especially if the latter has been overstained.

Figures 20--23 (Plate **4**) show a series of progressive changes in the centrosome; the centriole (*c'l.*) gradually becomes indefinite in outline and decreases in size until (Figs. 25–28, Plate **5**) one is unable to recognize it. The extent and form of the centroplasm (*c'pl.*) at its maximum development are best illustrated in Figure 21 (Plate **4**), which is from a section somewhat oblique to the long axis of the spindle, so that the centroplasm appears larger than it would have done had the section been perpendicular to this axis. This is the largest centroplasmic body that has come under my observation; its margin is very irregular and conspicuously crenate, being drawn out in the direction of the more conspicuous astral rays. The crenate condition of the centroplasm is characteristic of this stage. Later it becomes even in outline (Figs. 25, 28).

Figure 25 (Plate **5**) shows, at the inner pole of the spindle, a condition which I believe to be one of the phases through which the centrosome regularly passes: there is in this case no single body which we may describe as a centriole; however, there are to be distinguished in the centroplasm a number of very minute granules, which take a basic stain, and have a tendency to collect near the middle of the centroplasm. Figure 28 illustrates a similar condition. The stages represented in the two figures show that these granules have a slight tendency to an arrangement parallel to the axis of the spindle. In Figure 25 their axis makes only a small angle with that of the spindle, and in Figure 28 (inner pole) it is practically parallel to that axis. However, there is such a scattering of the granules that it is unsafe to attribute much importance to their arrangement. Furthermore, it is sometimes (outer pole of Figure 28) impossible to distinguish any granules in the centrosome. These eggs all show good fixation, and a satisfactory preservation of all the parts. At the outer pole of Figure 25 the granules are represented by a single body, — a typical centriole. This condition has been observed in a large number of eggs taken from different animals and treated with various stains. Boveri (:**01**) believes that the scattered condition of the centriole substance is due to poor fixation. Had this condition been observed in the eggs from only one or a few animals, I should hesitate to disagree with his interpretation; still, it seems as if Boveri were strangely inconsistent, for when he comes to describe a similar stage in the eggs of Echinus microtuberculatus, he shows (Taf. III. Fig. 27–31) that there are a number of minute particles distributed in the centroplasm, and that these particles gradually become less distinct until they almost disappear. In the next stage in Echinus these small granules have become a narrow band in the centroplasm, each end of which becomes the centre of one of the newly formed asters. This body increases in size and passes through a metamorphosis in the following cleavage generation similar to that just described. In his description, Boveri does not use the term "centriole," but the function of these granules is to give origin to a new centrosome; so that when we consider them in the light of their fate, it is permissible to apply to them the term "centriole." The interpretation of a body, it seems to me, should depend quite as much on its function as on its form. For this reason I think that we may use the term "centriole" in describing these changes in Haminea. The granules in the centroplasm in Echinus and in Haminea are similar, except that in Echinus they are more numerous.

The history of the centriole in Haminea up to this stage indicates that it appears under a variety of forms; and the succeeding changes, to be described in the next two sections, reveal still greater variations. Conklin (:**01**) finds that in Crepidula the centriole may be formed anew in the centroplasm (his medullary zone) in both maturation and cleavage stages. His observations on Crepidula and mine on Haminea suggest that Boveri's contention in regard to the independence and individuality of the centriole is open to serious question.

In Figure 26 the centriole is well formed and of considerable size; in the next stage (Fig. 27) it is larger, and the centroplasm, the margin of which has become more crenate, is more extensive. A section through the pole at right angles to the axis of a spindle in which the chromosomes are in a condition similar to those of Figure 27, shows the centriole in the form of a dumb-bell, the two bodies being not only close together, but still united to each other (Fig. 27 *a*).

If an attempt is made to correlate the changes in the centrosome with those in the chromosomes, it becomes evident that the relations of the two are not constant. The centrosome in Figure 28 (Plate **5**), for example, seems to have failed to develop as rapidly as usual. For while the centriole usually divides in the early anaphase of chromosome metamorphosis, in Figure 28 the centriole granules have not yet fused into a single body, — a change which must take place before the centriole divides. A number of similar cases have been observed, from which it may be concluded that the progressive changes in the centrosome and in the chromosomes take place more or less independently. However, we are able to find a connected series of changes in the centrosome which may be correlated in general with the changes in the chromosomes, and for this reason the series illustrated in Figures 19–29 (Plates **4**, **5**) is arranged in accordance with the known changes in the chromatin.

We may now inquire into the origin of the centroplasm. MacFarland ('**97**) explains the origin of the inner sphere (the centroplasm in Haminea) in Diaulula as resulting from a concentration of the substance surrounding the Centralkorn. In regard to this he says (p. 251) "Dem entsprechend ist auch die dichtere Substanz noch nicht zu annähernder Kugelform an den beiden Polen concentrirt, sondern besitzt etwa die Form flacher, planconvexer Linsen. Etwas weiter gehende Entfärbung ähnlicher Figuren bringt das Centralkorn im Centrum der Polsubstanz zur Anschauung, sowie einige Fibrillen, welche die beiden Platten verbinden." Lillie (:**01**, p. 239) says in regard to Unio, "The entire inner sphere [the centroplasm in Haminea] is the product of a single centrosome." He further says that the granules which mark the outer limit of this inner sphere are derived from the centrosome. In Crepidula (Conklin, :**01**), similarly to Unio, the medullary zone (the centroplasm in Haminea) is surrounded at first by four contiguous bodies which have themselves been derived from a single body, the centrosome. These four bodies fuse into a ring which marks the outer limit of the medullary zone. Here we have, then, three rather distinct methods of formation for the centroplasm, all of which lead to the same general result; that is, a centrosome differentiated into centroplasm and centriole.

The centroplasm in Haminea begins to appear while the eggs are in the ovotestis. In Figure 16 (Plate **3**) there can be distinguished immediately surrounding a central granule a very narrow area into which the rays do not penetrate. As soon as this area is distinguishable, the centrosome may be considered to consist of two parts, — centroplasm and centriole. As the centroplasm ("area") increases in extent, the centriole becomes irregular in shape. This irregularity continues during the growth of the centroplasm, which in Haminea seems to be derived entirely from the centrosome, as stated for other animals by MacFarland, Lillie, and Conklin. Here, however, it arises by a process different from that described by either of these authors. The centriole itself is as large in Figures 19, 21 (Plate **4**) as are the centroplasm and centriole together in Figure 16 (Plate **3**); that is, the process in Haminea involves an increase in the size of the centriole coincident with irregularities of outline.

Cortical Layer. The cortical layer (*st. ctx.*), a brief description of which has been given in a previous section (p. 267), shows no differentiation in eggs from the ovotestis, both primary and secondary rays extending to the centrosome; but in eggs that have been laid, the cortical layer exhibits two regions or zones. The inner zone takes a very light plasma stain, and only the primary rays extend through it to the centrosome (Plate **4**, Figs. 20, 21). This region will be referred to henceforth as the medullary layer (*st. med.*). It lies entirely outside the centrosome, and is not to be confounded with the term "medullary layer" as used by Conklin (:**01**) and myself (:**01**). The medullary layer in the sense in which I now use the term is differentiated from the "cortical layer" (see p. 267). In Figure 19 is shown a condition transitional between that of the cortical layer seen in Figures 13, 16 (Plate **3**), and that of the layer after the differentiation from it of the medullary layer (*st. med.*) as seen in Figures 21 *et seq.* (Plate **4**). Here (Fig. 19) the medullary layer takes a rather heavy plasma stain, and the secondary rays seem to terminate at its outer limit. In the following stages the medullary layer is clear, taking a very light stain, and it has no regular and sharply defined outer limit. In general form and extent it corresponds to the following conditions observed in other animals: for Diaulula MacFarland ('**97**) shows in Figures 29–32 (Taf. 20) a region which corresponds to the medullary layer in Haminea, and Lillie (:**01**, Plate 24, Fig. 9) figures for Unio a similar condition; the same region is named by Conklin (:**01**, Figs. A, 3, 3–5, and B, 3) cortical; and Boveri (:**01**, Taf. 5, Figuren 56–58) in describing it uses the expression "Zone medullaire," a term first used by Van Beneden et Neyt ('**87**).

The form of the medullary layer in Haminea varies considerably, owing to the extent of the encroachment of the cortical layer. When the outer pole of the mitotic figure reaches the periphery of the egg, the medullary layer becomes indistinguishable. Some of the changes involving variations in the outline of the medullary layer are illustrated in Figures 21, 23, 25, 27, 29 (Plates **4**, **5**).

Chromosomes. A cross-section of the spindle at the equatorial plane in the metaphase is represented in Figure 24 (Plate **4**); there are sixteen chromosomes, and between them can be seen the cross-sections of fibres, which appear as small dots.

While the egg remains in the ovotestis the chromosomes retain the tripartite form, which seems to be a resting condition; but as soon as it is laid, these irregular bodies tend to fuse into straight rods of nearly uniform calibre. A typical condition is seen in Figure 22 (Plate **4**), where the chromosomes are, in the main, straight rods, though slight irregularities are to be noticed in the outline of two of them, — the large chromosome lying on one side of the spindle, which has probably resulted from the fusion of two or more, and one of the narrow ones. This large chromosome would doubtless have become separated into two or more rod-shaped chromosomes before the first division occurred, had the egg been left to develop normally. However, the tripartite form may sometimes be retained until a comparatively late stage (Plate **5**, Fig. 25). In Figure 19 (Plate **4**) is shown one chromosome of a form that occurs very often at this stage, — four small masses of chromatic substance connected by a slender thread of chromatin. Chromosomes of this kind assume the rod-like form before division, and are always unusually long and thin. When we compare this form with the conditions seen in Figure 20, it seems probable that we have here a transition between the tripartite chromosome and the simple rod. Two of the chromosomes in Figure 20 indicate a possible relation to chromatin rings in tetrad formation, for they look as if they were about finishing a longitudinal division, the form being similar to that described by Griffin ('**99**) for Thalassema and Zirphea; but from the changes which follow, it is probable that this is not a case of longitudinal splitting, for in the first cleavage the chromosomes divide transversely (Fig. 29, Plate **5**). The chromosomes are at first connected by conspicuous chromatin threads, which become delicate, lose their capacity for stain, and finally disappear. The migration of the chromosomes toward the poles of the spindle takes place in the usual manner (Figs. 26–28).

(II.) Second Maturation Figure.

Considerable time was spent in attempting to bring into one series all of the stages observed in Haminea. The idea that two or more distinct processes might take place normally was thought to be improbable. Additional preparations exhibiting the second maturation figure were made, but the same differences were still found to exist. After further study it was found possible to bring the various conditions into two distinct series. The differences between the two are so great that they might well belong to distinct varieties of Haminea; indeed, one might question whether the eggs were not actually from different species, were it not for the fact that they were all laid, while under observation in the aquaria, by animals all of whose external characteristics pointed to their being of a single species. To the possible view that artificial conditions may have caused the difference, the reply can be made that the same differences have been observed in eggs collected from the regular breeding-places in the sea.

The two processes occur with about the same frequency, but all the eggs in one capsule follow either one method or the other. Any division of these processes into successive stages must be more or less arbitrary, especially since there are no periods of rest, the changes being both continuous and rapid. Until the centriole at the deep end of the first maturation spindle has divided, and the chromosomes of that spindle have begun to move apart, there is nothing to indicate that there are two processes, except possibly a difference in the size of the centrosomes. According to the degree of complexity of the changes which accompany this stage, and the length of time required for the metamorphosis, the processes may be designated as the shorter, or direct, and the longer, or indirect. The shorter process is characterized by the fact that the chromosomes remain free and separate from one another in the cytoplasm and by the direct formation of the achromatic figure; while in the longer process a vesicular nucleus is formed, the chromosomes becoming a part of a so-called quiescent nucleus, and the achromatic figure is formed indirectly. The shorter process will be described first.

(a) *Direct or Shorter Process.*

In the preceding section of this paper the changes in the first mitotic figure have been followed to the time of the transverse division of the chromosomes and the formation of the centroplasm and centrioles in the deep aster. The process now under consideration may be said roughly to begin with the anaphase of this stage. Figure 30 (Plate 6) shows a late anaphase of the first maturation figure. The chromatin in the polar cell has fused into one mass, and the centrosome cannot be seen. The centrosome at the inner pole lies close to the chromosomes, and owing to the obliquity of the section is in fact partly covered by them. The centrosome at this stage may lie so close to the chromosomes that it is impossible to be certain of its outline. In some animals it has been thought that the centrosome disappears entirely, — as has been claimed by Byrnes ('99) for Limax, — but in Haminea it may always be found when properly stained. The chromosomes are usually arranged in a circle, and in many instances the individual chromosomes are so close together that they seem to be fused; nevertheless, it is probable that they remain separate. The interzonal filaments are easily seen extending from one mass of chromatin to the other, and a small "midbody" is recognizable. The medullary layer of the sphere is thin and faintly outlined. A few rays remain attached to the boundary limiting the centroplasm, and within it are to be recognized two centrioles. The evidence of a spindle between the centrioles at this stage is very slight, so that none is represented in the figure. The whole centrosome at this stage is extremely faint and difficult to see.

In Figures 32 and 32*a* the first polar cell has been formed ; and the egg centrosome, now metamorphosed into the second maturation spindle with its two minute centrosomes, is seen lying nearly at right angles to the chief axis of the egg. The chromosomes within the egg are very small and lie outside the spindle, their relation to the spindle being indicated in Figure 32*a*. The crossing of the rays from the two asters is prominent on the side of the spindle nearest the animal pole of the egg. Intermediate stages between the conditions represented in Figures 30 and 31*a* have been observed, but they were unfavorable for reproduction. It may be stated, however, that the centrioles are connected by a central spindle, and that both the centroplasm and its limiting boundary, seen in Figure 30, disappear ; that the rays increase in length ; and that the sphere substance becomes indistinguishable from the surrounding cytoplasm, so that it has not been possible to establish any direct connection between the old sphere and the spheres belonging to the second mitotic figure. The successive positions assumed by the spindle, until it comes to be radial and to have one pole at the surface of the egg, are seen in Figures 32–36. In the case shown in Figure 34, the spindle evidently was formed more deeply in the egg than in the case of Figures 32 and 33.

The separation of the daughter chromosomes in the first polar cell is shown in Figure 32*a*.[1] The centrioles are very faint, and around each there is a diffusely stained region in which a few rays only can be seen. There can be distinguished in this polar cell 28 chromosomes, 14 in each half. It is probable that the other four lie beneath some of those counted. The derivatives of the first polar cell in Figure 53 (Plate **8**) show in an interesting manner the persistence of the interzonal filaments, and (what has been observed for these derivatives in this one case only) the presence of a small " midbody."

The centrosomes connected with the second maturation spindle grow each from a small granule, as seen in Figure 31 (Plate **6**), to a relatively large body composed of centroplasm and one centriole, as seen in Figures 33, 34, 36 ; but this enlarged condition is preceded by one in which the centrosome has an irregular form, as represented in Figures 32 and 35. The centrosome here seems to be elongated in a direction transverse to the axis of the spindle. During this change, it increases in size and in the intensity of its staining. The centroplasm arises in a manner similar to that described for the first maturation figure, appearing first as a thin layer closely enveloping the centriole. The outline of the centroplasm becomes somewhat crenate, but soon assumes an even appearance. The centriole, meantime, gradually becomes reduced in size and regular in form. The only difference between this centrosome and that of the first mitotic figure is that it is smaller than the latter. A sphere is evident, although not very distinct. The medullary and cortical layers may be distinguished in Figures 33 to 35.

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[1] An objection to considering the cell shown in Figures 32 and 32*a*, the polar cell of the egg shown in Figure 32, is the fact that it is more advanced toward an approaching division than the egg itself ; but this interpretation is confirmed by the relative positions of the two, and by the stage of development of the eggs associated with this one.

The chromosomes are drawn into the equatorial plane in a manner similar to that described by MacFarland ('97) for Diaulula and by Lillie (:01) for Unio. In Figure 32 they are vesicular; this seems to be their only approach to a so-called resting condition. In appearance they are identical with the chromosomes in the longer or indirect process, to be described presently, but the vesicular state occurs here much later than in the other process. This vacuolated condition gradually disappears, and the chromosomes again become solid and squarish or oblong. Their form is such that it is impossible to say whether division is longitudinal or transverse; they are seen in the act of separating in Figures 33 and 36. These changes are in many ways similar to those observed in Chætopterus (Mead, '98), Thalassema (Griffin, '99), and Cerebratulus (Coe, '99). From this point forward the two processes are not sufficiently different to require separate description.

Before passing to a description of the second or indirect process, the conditions represented in Figure 37 (Plate 6) may be noticed. Here we have a condition unusual for Haminea: other eggs found on the same slide as this one are in the metaphase and anaphase of the second maturation. The large cell lying near the spindle of the egg is probably the first polar cell. Its spindle is as perfect and clear as the one in the egg. The differences between the conditions in this case and those seen in the normal polar cell can be better appreciated by comparing Figure 37 with Figure 32 (compare also Figures 70 and 71, Plate 10). There is to be seen an accessory chromosome in the egg and another in the polar cell. The presence of an accessory chromosome in the polar cell may perhaps help ultimately in deciphering the meaning of this problematic body. Some observations made on the living egg in 1897 strengthen the view that this is the first polar cell. While studying the living egg, it was noticed that small cells containing deutoplasmic granules, and much larger than the usual polar cells, were occasionally formed at the time when the first polar cell ordinarily appears. These small cells were watched until the egg passed into the two-cell stage, which occurred at the usual time. There was so much albumen in the egg capsule that it was very difficult to follow the fate of the small cells with any certainty, so that I am unable to say positively that they divide, as the presence of the spindle in Figure 37 would lead us to expect; but the facts that they were produced in the living egg at precisely the time the first polar cell appeared in the surrounding eggs, and that the same conditions were afterwards found in preserved material at this stage, make it highly probable that we have here a very much enlarged polar cell. These observations support the generally accepted view, first advanced by Mark (**'81**), that the polar cells are abortive eggs.

(β) *Longer or Indirect Process.*

The second process or method of the formation of the second maturation spindle is more elaborate. The conditions in Haminea are usually so evident that there can be no doubt as to their occurrence. In this process the chromatic substance passes through a stage which resembles, more or less closely, the condition of the so-called resting nucleus; the chromosomes become surrounded with a nuclear membrane, the cavity of which is traversed by linin fibres, but the chromosomes do not lose their individuality.

One of the most noticeable facts in this process is the lack of synchronism between the changes in the centrosome and those in the chromosomes. A sequence based on the changes in the chromosomes would, for example, place such a stage as that represented in Figure 42 (Plate **7**) before the one shown in Figure 39. After the chromatin passes into the nuclear-vesicle condition, it remains for a certain period without change of form or position while the achromatic figure is undergoing a number of important alterations. Wilson (**:01**^{b}) has shown, by results obtained experimentally, that the centrosome may act independently of the chromosomes. The case of Haminea is the more interesting because in it the process is normal.

In describing this process, *the progressive changes in the achromatic figure* of the second polar spindle have been taken as the basis for determining the sequence of events.[1] If the conditions of the chromatin represented in Figures 42 and 43 are excluded, the changes in the chromosomes follow the usual order.

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[1] In selecting the progressive changes in the achromatic figure as the basis for determining the sequence of events, my purpose has been to emphasize the history of the changes in the second polar spindle.

So far as I have been able to observe, both the direct and the indirect processes have their inception in such a stage as is shown in Figure 27 (Plate **5**). Since in the morphology of the earlier stages there is nothing, as has been said, to indicate by which process the development will take place, there must be peculiar physiological conditions which determine the changes through which the egg passes; but at present it is impossible to state what they are. The two centrioles which accompany the second maturation spindle are already formed (Plate **5**, Fig. 27 *a*), and are surrounded by a layer of centroplasm. Stretching from centriole to centriole there is at all stages (Fig. 27; Plate **7**, Fig. 38) a definite central spindle, which increases in length as the centrioles move apart.

Plate **7**, Figure 38 (compare Plate **10**, Fig. 72) shows a condition which is typical for a fairly early stage in this indirect process. The centroplasm has become limited by a definite outer boundary, and from now on takes a very faint plasma stain; the primary rays are conspicuous, and terminate in the outer boundary of the centroplasm (*c'pl.*); the secondary rays are very faint and are gradually becoming shorter; the medullary zone (*st. med.*) around the deeper pole has become much larger than in the preceding stage; but at the outer pole it is wanting, and there is nothing to indicate that cortical and medullary layers have ever been differentiated there. The movement of the maturation figure toward the surface of the egg compresses the cytoplasm in this region; this in turn reacts directly against the centrosome, which thus becomes flattened. The outer centrosome never becomes as large as the inner one, and its rays are less conspicuous.

Some of the stages which I have incorporated in the indirect process belong, so far as the condition of the chromatin is concerned, earlier in the description; but since the changes in the achromatic spindle have been taken as the basis for determining the sequence, and since these stages have an intimate connection with the indirect process, they are included here.

The chromosomes in Figure 38 (Plate **7**) are arranged in two clusters: the outer one is destined to pass into the first polar cell, the inner to be contained within the nuclear vesicle, the wall of which has, indeed, already made its appearance. Already, too, the chromosomes are connected with the wall by linin fibres. In Figure 39 the surface of the egg at the animal pole has been raised up and the abstriction of the first polar cell has apparently begun, even though the chromosomes are still near the equator of the spindle.

It will be seen by comparing Figures 38, 39, and 49, that the axis connecting the new centrioles has no fixed direction in relation to the axis of the first maturation spindle. I have never seen a polar cell resulting from this second or indirect process of maturation while it was in the act of being constricted off, — a stage corresponding to that of the direct process illustrated in Figure 30 (Plate **6**); but the stages immediately before and immediately after the formation of the first polar cell by the indirect method are abundant.

The deep centrosome in Figure 38 may be looked on as a stage of long duration, because the eggs found in this condition far exceed in number those exhibiting conditions that precede or follow it. The changes following this condition take place rapidly. The first indication that the centrosome has entered on another phase is the occurrence of small breaks in its boundary (Figs. 39, 40). Immediately after this boundary begins to break down, faint indications of a new accumulation of cortical substance around the centrioles appear; usually this is first seen between the centrioles and the end of the centrosome; however, it may appear simultaneously on all sides of the centrioles. With the appearance of this new cortical layer there are formed rays which at first are short and few in number, as in Thysanozoön (Van der Stricht, **'98**). As soon as the rays are formed, the term "centrosome" should, according to our definition, be applied to the body which hitherto has been termed "centriole." The centrosomes, thus formed by the enlargement of the centrioles, now begin to separate from each other; the central spindle (*fus. c.*) becomes more conspicuous, and the cortical layer increases in extent (Figs. 41–45). The growth of this central spindle is interesting; it is limited laterally by a well-marked boundary; within this there are several fibres extending from one centrosome to the other (Plate **7**, Figs. 42, 44; Plate **11**, Fig. 74). As the spindle increases in length, the outer boundary increases in distinctness and the contained fibres become less evident; this differentiation continues until the fibres become very faint and at the same time anastomose (Fig. 45) with one another. This gives them a branched appearance, similar to the conditions found in Diaulula by MacFarland.

The next stage is represented in Figures 46 (Plate **7**) and 76 (Plate **11**). There extends between the two asters a conspicuous fibre. This is probably the remnant of the central spindle; if so, it has increased in staining capacity. It has an irregular, wavy course; the outer end approaches as near the animal pole as the centrosome, but terminates at one side of that structure in the outer edge of the cortical layer of the sphere; the inner end bifurcates, one branch being directed toward the centrosome, the other lying tangent to the cortical layer of the sphere. The three ends of this fibre terminate in the dense cortical layer, so that it is difficult to determine its exact extent.

The cytoplasm has now encroached on the region formerly occupied by the spindle; the chromosomes do not lie in the section containing the two centrosomes. It will be noticed that in Figure 45 the spindle fibres have a different origin from the astral rays, since the spindle fibres arise from the centrioles, but the rays from the cytoplasm. In Figure 46 the astral rays have not formed on the side toward the spindle, as they usually do. The large fibre seen in Figure 46 breaks up and disappears in the cytoplasm. With the disappearance of this fibre, the centrosomes, which may be assumed to have been held together by it, quickly separate, the deeper one moving most. The outer aster, in fact, moves only a little nearer the periphery (compare Plate **7**, Figs. 46 and 47; Plate **11**, Figs. 77 and 79), whereas the inner one migrates a considerable distance into the egg, causing a movement of the deutoplasm which is thereby excluded from a long, narrow region that will soon be occupied by the nascent spindle.

During the separation of the asters, the cortical layer (*st. ctx.*) of the sphere has increased in extent and definiteness. The primary astral rays have increased in length and number, but there is as yet very little evidence of secondary rays. The wall of the old centrosome, as has been said, has gradually broken up into small particles, which can no longer be distinguished from the granules in the cytoplasm (Figs. 41–44). During these changes the size of the old centrosome increases greatly, but the staining reaction of the portion not occupied by the spindle remains the same (Fig. 38). As the old wall disappears, the surrounding cytoplasm approaches the maturation figure until it comes into direct contact with it. The behavior of the cortical and medullary layers shows how intimately these are associated with the centrosome. The cortical and medullary layers either increase in size so as completely to surround the new achromatic figure (Plate **7**, Figs. 42, 43; Plate **9**, Fig. 58; Plate **11**, Fig. 73), or they may be confined to the inner pole (Fig. 45), in which case they are not as large as in Figure 38. On the other hand, both layers may disappear at an earlier stage.

Figure 41 (Plate **7**) shows the fate of the astral rays of the parent centrosome; for in addition to the fine short rays centring in the new astral figures, there are seen to be in the cytoplasm surrounding the disintegrating wall of the old centrosome a number of rays and rows of granules arranged radially to the old centrosome. In some instances the granules are larger than those of the cytoplasm ; in others they are of about the same size. Whatever may have been the origin of the rays of the first maturation figure, their fate is unquestionable, since they break up into granules which finally become indistinguishable. The relation of the old and new astral rays to each other, and the relation of the old rays to the sphere-substance, are well illustrated in the somewhat unusual stage shown in Figure 58 (Plate **9**), which is described beyond.

Chromosomes. The earliest stage in the metamorphosis of the chromosomes, in the indirect process, is found in Figures 39 and 42 (Plate **7**). The conditions represented in Figure 39 are similar to those seen at this stage in the direct process and in other animals generally. The chromosomes have divided by a transverse division, and some of them have begun to migrate toward their respective spindle poles. In Figure 42 there is to be noticed, besides the regular chromosomes lying in pairs, an accessory chromosome that has already become enclosed by a vesicle of its own, three sides of which are plainly discernible, as are also linin fibres extending from the chromosome to the wall of the vesicle. In Figure 38 the two masses of chromosomes have moved apart ; and around the egg chromosomes a faint wall, connected with them by delicate linin fibres, is visible. In this egg the nuclear vesicle evidently was completely formed before the chromosomes had changed much in position. In Figure 43 each of the chromosomes has the form of a minute, thick-walled vesicle, and is still free in the cytoplasm, no nuclear wall having yet been formed around them. In Figure 40, on the contrary, the chromosomes, though already vesicular, are enclosed in a common, well-formed nuclear wall, the linin fibres have become distinct, and the chromosomes are again assuming the solid condition, owing to a gradual thickening of their walls. The chromosomes do not assume the form of large vesicles which fuse to produce the wall of the nucleus, as in the telophase of the second maturation figure, but retain their individuality during the formation of the nuclear wall. The vacuolated condition of each chromosome seems to be a phenomenon which is not necessarily associated with the formation of a nuclear vesicle. The linin threads grow from the chromosomes to the wall, and not *vice versa*, as is shown by the fact that there are threads which are attached to the chromosome, but have not reached the wall, whereas the converse of this was never found. It is evident that considerable variation exists as to the time when the nuclear membrane forms; also as to the time when the chromosomes pass through the vesicular stage.

The chromosomes may be grouped into two or more nuclei. In Figure 41 (Plate **7**) there are two well-defined nuclei, and at the side of the achromatic figure is the outline of a thin-walled vesicle; however, no chromosomes could be found in the vicinity of the vesicle in adjacent sections, nor were there any linin fibres to be observed within or around it. In a second instance the chromatin was found to have assumed the form of two quiescent nuclei before the achromatic figure had begun its peripheral migration, — a fact which would seem to indicate that the formation of more than one quiescent nucleus is independent of the movement of the achromatic figure.

In the quiescent nucleus the chromosomes remain separate. Before the membrane surrounding them has disappeared and before the spindle rays have come into contact with them, they divide; but the rod-like form is not assumed before division. It would be impossible to say with confidence that the division is exclusively either longitudinal or transverse. In judging whether the division of the chromosomes is longitudinal, one has to be governed by their form. In Haminea the chromosomes are squarish in outline; but before the division the square form is usually exchanged for an oblong one, and the daughter chromosomes by the transverse division of the oblong chromosomes become small squares.

When the chromosomes divide (Fig. 48), there appear fine close-set fibres, which grow from the chromosomes in the direction of the two asters till they reach the nuclear membrane (Plate **7**, Figs. 47, 48). These fibres continue to grow after the nuclear membrane breaks down (Figs. 49, 54) until they reach the region of the centrosome (Plate **8**, Figs. 54, 55). These finer fibres are easily distinguished from the linin fibres in the nucleus (Plate **7**, Fig. 48; Plate **8**, Fig. 55); they are not formed from the free linin fibres, but are new outgrowths from the chromosomes.

Astral rays. After the central spindle disappears, the asters are usually located on opposite sides of the nucleus (Plate **11**, Fig. 77). There are no rays which are continuous from aster to aster, nor are there at first any connecting the aster to the nucleus (Plate **7**, Fig. 48). In the formation of the definitive spindle the cytoplasmic granules in the territory between the asters and the nucleus arrange themselves into rows, which soon assume the appearance of continuous fibres. These new fibres reach on the one hand to the nuclear membrane, and on the other to the cortical layer of the sphere. When they begin to be formed (Fig. 47), they are not strictly radial, but often are more or less curved in various directions independent of the form of the prospective spindle. Sometimes (Fig. 48) the fibres between the nucleus and each of the asters are so curved in relation to one another as to produce a short truncate or cask-like spindle, such as is seen in plant cells, and sometimes in animal cells, during nuclear division. Each of these two cask-like bundles of fibres later contributes to the formation of the spindle. These fibres are purely cytoplasmic in origin, and continue to be formed until the new spindle is complete. As the fibres increase in number, the nuclear membrane breaks down, and the fibres become attached to the chromosomes. During this process the fibres do not push before them the nuclear membrane, as in cleavage, but the outline of the nucleus remains regular as long as it is discernible. When the two asters and the nucleus do not lie in a straight line (Plate **7**, Fig. 49; Plate **11**, Fig. 79), the direction of the forming fibres is noticeably modified.

In Figures 55 (Plate **8**) and 80 (Plate **11**) the mitotic figure is in the metaphase. The spindle at this stage is very long, but it becomes much shorter during the anaphase, when the separated chromosomes move toward the poles of the spindle (Fig. 50). This shortening of the spindle sometimes occurs before the chromosomes have begun to move apart.

Centrosome. Nearly at the same time with the beginning of these changes in the chromosomes, and during the initial stages in the formation of the spindle, the centrosome has become differentiated into centroplasm and centriole (Plate **7**, Fig. 47). The lack of correlation, not only between the progressive changes in the centrosome and those in the chromosomes, but also between the two centrosomes of a single spindle figure, is evident from the drawings.

As seen in Figure 46 (Plate **7**), each centrosome at the time of the disappearance of the central spindle is a solid, spherical, deeply staining body. The centrosome at the inner pole of Figure 47 shows a thin layer of centroplasm surrounding an irregular centriole, but the one at the outer pole of the same egg remains undifferentiated. In Figure 48 the two centrosomes exhibit similar differences, while in Figure 49 both centrosomes are solid or undifferentiated. In Figure 54 (Plate **8**) centroplasm and centriole are already clearly distinguishable in both centrosomes; but in Figure 55 the outer centrosome is still homogeneous. The centroplasm arises from the substance of the centrioles precisely as in the case of the centrosome of the first polar spindle. During these changes the centriole remains irregular in outline until the centroplasm is well differentiated, whereupon it becomes smaller and spherical. The intimate relation between the differentiation of the medullary and cortical layers of the sphere, on the one hand, and that of the centrosome and centriole, on the other, is noticeable in the indirect, as it was in the direct process; in every instance, with the one exception of the outer pole of Figure 55, the medullary layer is not distinguishable until the centroplasm can be seen. It is at this period that the secondary rays of the asters become numerous.

The changes in the indirect process have now been followed from the first indication of the achromatic figure until the completion of the second maturation spindle. The chromosomes of the second spindle have divided (Fig. 55), but have not begun to migrate toward its poles, and the achromatic figure has reached about the same stage of development as the one last described (Plate 6, Fig. 36) in the shorter process. The further maturation phenomena are the same in both processes, and hence may be described together. In these changes there is nothing to indicate whether the egg has passed through the direct or indirect process. A comparison of Figures 36 and 37 (Plate 6) with Figures 54 and 55 (Plate 8) shows that a difference in size is the only real distinction. If the long spindle of the indirect process did not become shortened, it might serve as a distinguishing feature.

(c) *Further Changes in Maturation.*

The further maturation changes may be briefly described as follows: In Figure 50 (Plate 8) the divided chromosomes have moved toward their respective poles, and the interzonal filaments stretching between them are easily seen. The centrosome at the inner pole is clear and sharply outlined; it has become somewhat compressed in the direction of the axis of the spindle. The centrosome at the outer pole is small and apparently much flattened in the same direction as the one at the inner pole; but it lies so close to the periphery of the egg that it is difficult to determine its outline. There are a number of large, deeply staining granules scattered about in the cytoplasm surrounding the deep centrosome which were not before distinguishable.

The next older stage illustrated is shown in Figure 52. The chromosomes have moved apart still farther, and indeed have come so close to their respective centrosomes as partly to cover them. The animal pole of the egg has been raised up into a prominent conical elevation, near the apex of which the outer centrosome and its cluster of chromosomes are located. The latter are more closely crowded together than the corresponding bodies near the inner or deep centrosome. The interzonal filaments are faint and irregular. The centrosomes have likewise become faint, and the centrioles, though distinguishable, no longer take a very dark stain.

At the next stage shown (Plate **12**, Fig. 83) the centrosome of the outer pole has disappeared, and the one at the inner pole is less distinct than in the preceding stage. The medullary and cortical layers of the sphere within the egg are still present, though not sharply defined. Each chromosome now becomes hollow; its walls are at first thick and take a heavy stain; but as the vesicle grows, its walls become thinner. These nuclear vesicles lie in a cluster around the fading centrosome (Fig. 84). The centrosome may be recognized until the vesicles begin to fuse together to form the female pronucleus (*pronl.* ♀) when the boundary of the centroplasm becomes wrinkled and shrunken, and no centriole can be distinguished.

In some eggs, not reproduced, persistent rays pass between the vesicles formed from the chromosomes, and though they radiate from a common point, there is no recognizable body at that point, the centrosome having completely disappeared. As the vesicles begin to fuse into one large vesicle, small particles of chromatin make their appearance within the vesicles. The female pronucleus (*pronl.* ♀), resulting from this fusion of vesicles, is irregular in outline when first formed (Plate **8**, Fig. 53; Plate **12**, Fig. 85), and larger than the male pronucleus (*pronl.* ♂). During the fusion of the vesicles to form the female pronucleus the astral rays break down and disappear in the cytoplasm, although a few may be present as late as the stage shown in Figure 85. The female pronucleus is usually surrounded by a layer of fine granules, the outer limit of which merges imperceptibly into the surrounding cytoplasm. This is the substance of the medullary layer of the sphere, which has now spread itself around the female pronucleus. This condition is similar to that observed by Conklin (:**01**, p. 283) in Crepidula, but is unlike the condition described by Lillie ('**97**, p. 245 ff.) for Unio. However, this sphere-substance does not follow as definite a course of changes in Haminea as it does in Crepidula.

A few unusual conditions have been found in Haminea. Two of these are represented in Figures 58, 59 (Plate **9**). In Figure 58 the outer limiting boundary of the parent centroplasmic mass is present, and extends about halfway around the new achromatic figure. The line is apparently continuous, and yet the new astral rays pass through it. The gradual disappearance of the sphere-substance and the encroachment of the cytoplasm is thus well illustrated. The new cortical layer about the new centrosomes is similar in form and extent to the centroplasm of Figure 54 (Plate **8**). But that it is not centroplasm in this case is shown when the stain is nearly all removed, for then the rays may be traced to the central body, — the undifferentiated centrosome. This is the only case observed in which the wall of the old centrosome persisted in any such form as this. Eggs have been found (Plate **11**, Fig. 73) in which the achromatic figure of the second spindle was as far advanced as the one represented in Figure 58 before the first polar cell had formed.

In my preliminary paper (Smallwood, :**01**) on Bulla [Haminea] the conditions represented in Figure 59 were described as belonging to the process of fertilization. The egg at that time had a heavy hæmatoxylin stain. Since then it has been decolorized and restained in Brazilin; there are now to be recognized deep in the egg seven vesicles, from which numerous rays extend in all directions. The rays all centre at one point. Deutoplasmic spheres are found completely surrounding the vesicles and their rays. The remains of the second maturation spindle are seen at the animal pole of the egg immediately beneath the first polar cell. The chromosomes are small and do not take a heavy stain. No fibrous connection could be traced between them and the rays extending from the vesicles. This stage shows a very unusual variation in the development of the first polar cell and the conditions during the telophase of the second maturation spindle. It has, however, nothing to do with fertilization.

The maturation changes in Haminea may show considerable variation. It is probable, so far as one may judge from the appearances of the egg, that in these two cases (Figs. 58, 59) the egg would have segmented. It has hitherto been assumed that maturation takes place with a great deal of regularity; but in Haminea there is certainly variability, for not only are there two distinct processes in the second maturation phenomena, but at some of the stages in each of these processes there is also variation.

(d) *Literature and Discussion of Results.*

With the appearance of Boveri's (:**01**) work on the centrosome, it is probable that there will be no longer such strong opposition to the application of the term "centrosome" to the body which occurs at the centre of the aster, — the body, whatever its size and form may be, in which the primary astral rays terminate. Large centrosomes have not been described in many species of animals, but they seem to be characteristic for Mollusca. The following observers have described large centrosomes, or bodies to which the term "centrosome" is applicable: Mark ('81) in Limax, Boveri ('90) in Heteropods, McMurrich ('96) in Fulger, MacFarland ('97) in Diaulula, Murray ('98) in Pulmonates, Conklin ('98, :01) in Crepidula, Byrnes ('99) in Limax, Linville (:00) in Limax and Limæa, and Lillie (:01) in Unio. The centrosome in Physa (Kostanecki und Wierzejski, '96) is probably the same as in other Mollusca.

The fact that the centrosome may pass through a cycle of changes has been recognized only recently. If the various observers cited had all had this possibility in mind when carrying on their studies, it is probable that there would have been more agreement in their results. Large centrosomes have been observed in other phyla by the following workers: Korschelt ('95) in Ophryotrocha, Wilcox ('95) in Caloptenus, Klinckonström ('97) in Prostheceræus, Van der Stricht ('98) in Thysanozoön, Van Name ('99) in Planarians, Gathy (:00) in Annelids, and Boveri (:01) in Ascaris and Echinus. Wilson (:00), in summarizing the results of various investigators, is inclined to favor the restricted use of the word "centrosome," limiting it to the body termed centriole in this paper. On page 314, he says: "By following Boveri's terminology, therefore, MacFarland is driven to the strange conclusion that the second polar spindle is nothing other than an enormously enlarged 'centrosome,' — a result little short of a *reductio ad absurdum* when we consider that in Ascaris the polar spindle arises by a direct transformation of the germinal vesicle." Notwithstanding this criticism, I believe that the term "centrosome" as defined by Boveri should be accepted; and the absurdity disappears when we recognize the growth and metamorphosis which this body exhibits.

In a previous part of this paper the progressive changes in the growth of the centrosome in Haminea have been mentioned, and the manner in which the centroplasm was probably formed has been described. We may now discuss the derivation of the centrosomes of the second mitotic figure from the deep centrosome of the first maturation figure. MacFarland believes that, in the formation of the second polar spindle (Diaulula), the centrosome divides and that the new centrosome results from a new arrangement of the parts. The inner sphere (the centroplasm in Haminea) in his opinion is formed by a process of condensation around the "Centralkorn" (centriole) and persists in the new centrosome. Boveri (:01), on the contrary, maintains that the whole centrosome divides, its division being initiated by the division of the centrioles. He further believes that all centrosomes may be shown to divide in one or the other of the four typical methods which he figures in his text (pp. 102, 103). In Crepidula (Conklin, **:01**), however, there is positive evidence against this view, and the changes in Haminea do not fall under any of his four typical methods. In Figures 41, 44 (Plate **7**) the wall limiting the centroplasm may be seen ; it gradually breaks up into small granules, which merge in the cytoplasm. The new centrosomes are minute, solid, undifferentiated bodies ; they are not at once surrounded by a layer of centroplasm that is limited by a continuous line, but there is formed about each new centrosome a new cortical layer of sphere substance. The centroplasm does not appear until later; and when it does appear, it is a new formation, not a product of the old centroplasm. The centroplasm of the centrosome in such a stage as is represented in Figures 38 and 39 (Plate **7**) finally becomes indistinguishable, being diffused in the cytoplasm (Fig. 45). A part of the old centroplasm may be directly transformed into the cortical layer of the new sphere, but there is no staining reaction in it until breaks appear in its limiting wall. While the following description by Boveri of the division of the centrosome in Ascaris may be true for that species, it certainly finds no confirmation in Crepidula or Haminea. Boveri (**:01**, p. 98) says: "Hier streckt sich das Centrosom in der Richtung der Verbindungslinie der beiden Centriolen in die Länge, und um jedes Centriol schnürt sich die Hälfte des Centroplasmas ab. Die Substanz des Muttercentrosoms schient ganz oder fast ganz in die beiden Tochtercentrosomen aufzugehen, die sich alsbald zu Kugeln abrunden und nun wieder von neuem heranwachsen."

Mark (**'81**) was one of the first to call attention to the importance of ascertaining the origin of the second achromatic figure, but not until 1894 was there much definite evidence on this problem. At this time Heidenhain (**'92**) stated that the centrosome gave rise to the whole central spindle. Since then have appeared observations by the following persons who sustain this conclusion: MacFarland (**'97**) in Diaulula, Van Name (**'99**) in Planarians, Van der Stricht (**'98**) in Thysanozoön, Lillie (**:01**) in Unio, and Conklin (**:01**) in Crepidula. In Haminea (Plate **7**, Figs. 38–45), the formation of the achromatic figure in its early stages agrees substantially with the conditions in the cases cited ; but Haminea differs from all of them in the breaking down of the central spindle and the formation of a new one. The formation of a spindle, which afterward disappears, has been observed by Griffin (**'99**) in Thalassema, by Coe (**'99**) in Cerebratulus, and by others ; but in all of these cases the spindle disappears before it has become as large as it is in Haminea. A definite spindle is always present between the centrioles in the early stages of the longer or indirect process, and usually in the shorter process also, — a condition which differs from that found by MacFarland and Boveri (:01), who hold that in Diaulula and Ascaris it is formed only after the centrioles have moved apart a certain distance; but it is in agreement with the account given by Conklin (:01) for Crepidula, although in the latter case the centrioles themselves originate in a different manner. When the cytoplasm that immediately surrounds the achromatic figure is studied (Plate 7, Figs. 46–49), no specifically differentiated substance is noticeable even with high powers of the microscope. The new spindle (mantle fibres) arises from the cytoplasm in this region; the granules which go to form the fibres may begin to collect into lines before there is any direct physical connection with the centrosome. The cytoplasm seems to take an active part in causing these new rays to form, as if it had a ray-creating power. Morgan (:00, p. 504) says: "The protoplasm, under the action of strychnine, produces fibres that fill the egg. . . . All the rays converge to the nucleus as a center." Boveri ('88) maintained that the granules were formed into rays, but that they were aggregated about the centrosome. The appearance of rays in Haminea is preceded by that of a number of distinct granules arranged in rows. It has already (pp. 288, 289) been pointed out that the astral rays become granules as they undergo disintegration. In the growth of the new rays the process is reversed.

The spindle fibres, which arise in the cytoplasm, I have termed "mantle fibres," because they are most abundant at the periphery of the fine fibres which grow out from the chromatin. The derivation of the mantle fibres from the cytoplasm agrees with the observations of Hermann ('91), Moore ('95), MacFarland ('97), and others. The fine fibres originating from the chromatin are surrounded by the mantle fibres, and, I believe, take the place of the central spindle that has disappeared. Agassiz and Whitman ('89, Plate 24, Figs. 1, 2, 3; Plate 27, Figs. 1, 4) represent for osseous fishes conditions of the spindle that are similar to those of Haminea (Plate 7, Fig. 49; Plate 8, Fig. 54). The complete second maturation spindle, then, is composed of cytoplasmic mantle fibres and an "axial" spindle, the fibres of which are derived from the chromatin.

One might at first thought imagine that the centrioles would pass through similar phases in both the first and second polar spindles. Such, however, is not the case. The centriole in the deep centrosome of the second maturation figure is completed when the centroplasm is formed, whereas in the first maturation the deep centriole might be termed the *Anlage* for the whole of the young achromatic figure of the second maturation.

Chromosomes. In the indirect process in Haminea there is a definite telophase following the formation of the first polar cell. The wall of the nuclear vesicle in the cases observed (Plate **7**, Figs. 38, 40–45, 47, 48) was formed from substance that was destitute of the granular constituents of the rest of the cytoplasm. This differentiated substance, in which the spindle and chromosomes lie, has been described under various names, — archoplasm, karyoplasm, hyaloplasm, etc.; it is from this substance, whatever called, that the wall of the nucleus is produced.

The formation of a nucleus between the first and second maturation has been observed to occur in other animals, as follows: in bony fishes by Kupffer und Benecke (**'78**); in Petromyzon planeri by Boehm (**'88**, p. 637), who terms this nucleus "eine provisorische Eikern;" in pulmonate molluscs by Garnault (**'88–89**, p. 13), who terms it "noyau vésiculeux intermédiaire;" in molluscs and Echinus microtuberculatus by Boveri (**'90**, p. 38), who describes it as a "bläschenförmige Kern;" and in Allolobophora fœtida by Foot (**'97**), who found it occurring in one instance. The appearance of this nucleus in Haminea is similar to that shown by Boveri (**'87**, **'88**) in the telophase of the second maturation in Ascaris megalocephala. In Ascaris the chromosomes at first remain distinct, but they become connected with the wall of the vesicle by linin fibres, just as they do in Haminea. Moore (**'95**) found that in Elasmobranchs, after the close of the first and second spermatogenic periods, the chromosomes came to lie in vacuoles. Sutton (**:00**) finds that in the spermatogonial division of Brachystola each chromosome may be converted into a separate vesicle. And, finally, in the spermatogenesis of Paludina (Meves, **:01**), each chromosome becomes vacuolated, and may either remain distinct or become fused with others to form an irregular nucleus.

In Haminea the chromosomes divide before the mantle fibres penetrate the nucleus. The division in this case seems, therefore, to be an act independent of the mantle fibres.

A quantitative reduction of the chromatin is evidently accomplished by the formation of the two polar cells; but it is difficult to imagine how any qualitative reduction can have taken place. Recent results in experimental cytology tend to show that the individuality of the chromosomes has not been established for all cases. It has been shown in Haminea (p. 231 ff.) that the chromosomes pass through stages which must involve a complete structural rearrangement of their material. If there is to be a subsequent qualitative reduction, some kind of a symmetrical arrangement of this material is necessary in order that there may be subsequently an equal division; but the forming chromosome usually becomes irregular in outline, rendering improbable a symmetrical arrangement of its constituent elements.

So far as I know, the existence of two processes or methods in maturation has never been observed in other animals, except that Lillie (:01) found in one instance that in Unio the egg exhibited two quite distinct processes in the formation of the second mitotic figure. In explanation of this phenomenon he says (pp. 243, 244): "The modification was undoubtedly due to some slight difference in the conditions of the cytoplasm in the two cases. That such different courses of events can yet lead to the same typical results indicates a most remarkable power of self-regulation of the entire ovum, and at the same time demonstrates that the mechanical processes of spindle and aster formation are not the primary factors in determining the position of the maturation spindles." The striking fact in Haminea is that the direct process recurs in nearly as many eggs as the indirect, both accomplishing the same result, — the formation of the second polar cell.

3. Fertilization.

In Haminea, after copulation takes place, the hermaphroditic duct is full of spermatozoa, which are most abundant in the lower and larger part of the duct, but may be found even in its branches.

The eggs and spermatozoa of the same individual are often found together in the follicles of its hermaphroditic glands. That these spermatozoa are mature, or nearly so, is to be inferred from the fact that they are all discharged during copulation, sections of such an ovotestis made after copulation showing only quite immature spermatozoa in these follicles. Nevertheless, self-fertilization does not take place, so far as I have been able to determine, since no sperm heads are found in the egg between the time when copulation takes place and the time when the egg passes into the hermaphroditic duct.

My studies on the spermatozoön have not been at all exhaustive. The mature spermatozoön (Plate 9, Fig. 60) is composed of the two parts, head and tail. The head is cylindrical, flattened, and of nearly uniform thickness, terminating rather bluntly at each end; it is distinctly bent near the middle. The portion anterior to the bend is somewhat shorter and more pointed than the part behind the bend. The tail is long and filiform. I was not able by the use of Delafield's hæmatoxylin and picric acid to differentiate a middle piece in the adult spermatozoön, nor have I given special attention to studying the ultimate structure of this element.

As the egg on being deposited passes through the hermaphroditic duct, the spermatozoön, it must be assumed, approaches and penetrates it (Plate **9**, Fig. 60; Plate **8**, Fig. 56), the tail being lost in the act. While the egg remains in the hermaphroditic duct, no change is noticeable in the sperm head, but immediately after deposition the head becomes much enlarged. Finely granular cytoplasm collects around it, and on one side of it there appear a few coarse granules; it was impossible, however, to trace the origin of these to any particular part of the spermatozoön. They persist for a considerable time, but neither they nor anything resembling a sperm sphere or sperm aster become permanently associated with the sperm head during its migration in the egg. The sperm head increases in size and becomes more or less elliptical in outline, though one end is usually more pointed than the other (Plate **8**, Fig. 57; Plate **9**, Fig. 64). It is very difficult, however, to say with any certainty which end of the sperm head leads during its changes in position. While the egg remains in the hermaphroditic duct, the sperm head usually lies near the periphery with its long axis at right angles to the radius of the egg; when migration begins, one end turns toward the deeper aster of the maturation figure. In some cases (Figs. 56, 62) the anterior end seemed to lead; in other cases the reverse was true. In still other instances the sperm head remained near the periphery of the egg until it had assumed a spherical form, the egg having meantime passed into the anaphase of the second maturation. While the sperm head is migrating through the deutoplasm, its outline shows all gradations of form from that of a much elongated oval to that of a sphere. The sperm head may remain solid and homogeneous until it has penetrated deep into the egg (Plate **8**, Fig. 57), — until the metaphase or even the anaphase of the second maturation (Plate **9**, Fig. 61); but it may show signs of vacuolation as early as the metaphase of the first maturation. The first evidence of vacuolation results from the central region not staining as densely as the rest of the head. The vacuoles are at first very small, and as many as six have been observed. There may be a central vacuole which increases in size, or several independent vacuoles may unite to form a large one (Figs. 63, 64). There is, therefore, no very precise correlation between the times of migration and vacuolation, nor between the metamorphosis of the sperm head and the maturation of the egg. The sperm head shown in Figure 61 is elongated and slightly constricted (cf. Limax agrestis, Byrnes, '**99**, p. 213, Pl. xi. Fig. 4). It still lies near the periphery of the egg, though the second maturation spindle has been formed. On the other hand, the sperm head represented in Figure 63 lies somewhat deeper in the egg than in the case of Figure 61, is swollen and nearly spherical, and already contains a single vacuole of irregular form and ill-defined outline, although the egg is only in the anaphase of the first maturation. These two cases illustrate sufficiently the lack of correlation between the development of the sperm head and the maturation of the egg. A considerably more advanced stage in the metamorphosis of the sperm head is seen in Figure 84 (Plate **12**). The head has now assumed the appearance of a vesicular nucleus, and has moved into the region near the animal pole which is destitute of deutoplasm; it has a thick, deeply staining wall, and is considerably larger than in the stage last mentioned; it contains, in addition to linin threads and chromatin, a homogeneous ground substance which stains very faintly. Further changes in the male pronucleus are represented in Figures 53 (Plate **8**) and 85 (Plate **12**). The wall is no longer thick, but it still takes a light stain, and may become irregular in outline; the linin fibres have united to form a scanty, coarse-meshed network, on which are located the chromatin masses. In one instance the male pronucleus had already assumed a condition similar to that of the female pronucleus shown in Figure 51 (Plate **8**), while the second maturation figure was only in the telophase.

From the time that the spermatozoön enters the egg until the anaphase of second maturation, there is no evidence of an aster in the egg aside from those associated with maturation. The progress of the spermatozoön through the deutoplasm is unattended by any evidence of radiation. In three instances, during the late anaphase of the second maturation, an aster (not figured) has been found in the centre, or very near the centre, of the egg. In no case could any connection be made out between these asters and the sperm head.

4. Early Cleavage.

(a) *Metamorphosis of the Chromatin.*

Since there seem to be no essential differences in the process of metamorphosis in the female and male pronuclei, the earlier changes in the sperm head (p. 300) excepted, I shall confine my description to the female pronucleus.

The first differentiation of chromatin is observed when the vesicles, which arise from the chromosomes in the telophase of second maturation, prepare to fuse together to form the female pronucleus. When these vesicles begin to unite, linin threads appear, and small granules of chromatin are found on them (Plate **12**, Fig. 85). A distinct increase in the volume of chromatin follows, and several plasmosomes appear; all of the chromatin, as distinguished from the plasmosomes, gathers on the linin threads, and the result is the deeply staining, typical spongework characteristic of the quiescent nucleus (Plate **8**, Fig. 51).

The next step in the metamorphosis of the chromatin is the breaking up of the extensive spongework into isolated masses, which in turn are resolved into spherical bodies, either grouped or single (Plate **12**, Fig. 88). Many of the spheres of chromatin are further changed into ring-shaped bodies, which are connected with one another by linin threads (Plate **12**, Fig. 87; Plate **13**, Fig. 89); the chromatin bodies which are not thus metamorphosed become hollow spheres (Plate **12**, Fig. 86; Plate **13**, Figs. 90, 93). The solid and the vacuolated chromatin bodies form the chromosomes of the first cleavage spindle, while the remainder of the chromatin (including the plasmosomes) disappears, either immediately before or after the nuclear wall breaks down (Plate **12**, Figs. 81, 86, 87; Plate **13**, Figs. 89, 90, 93). In one nucleus (Fig. 89) there are two large masses of chromatin which have not yet broken up into spheres.

(b) *Origin and Fate of the Cleavage Asters.*

The centrosome at the deep pole of the second maturation spindle disappears when the chromosomes fuse to form the female pronucleus. From this time until the chromatin has broken up into spherical bodies (Plate **12**, Fig. 88) there are no distinguishable centrosomes in the egg. The earliest observed condition of the first cleavage centrosome (Fig. 88) shows two distinct and widely separated asters, unconnected with each other; each is associated with one of the two pronuclei. In this case a large portion of the upper nucleus was removed with the section preceding the one figured. The aster nearer the surface of the egg is the deeper in the section and has already begun to push in the wall of the larger (deeper in the section) pronucleus. The other aster lies at a high level in the section and has its rays associated with the small portion of the upper pronucleus that in cutting fell to this section. Another fairly early condition in the formation of the cleavage spindle is shown in Figure 87. The outline of the pronucleus uppermost in the section coincides with that of the lower one, so that it is difficult to distinguish one from the other; but in the region of the asters, there is evidence that only one of the pronuclei is closely associated with each aster; the left aster in this case is associated with the upper pronucleus, while the right aster is associated with the deeper one. There are present a few more rays than in the previous stage (Fig. 88), but no distinct central spindle is yet formed. In Figure 90 (Plate **13**) the outline of the upper nucleus is somewhat smaller than the lower one, so that the former extends beyond that of the latter except at one place. The central spindle is partly formed, and the relation of the asters and the forming spindle to the two pronuclei is clearer than in the preceding stage. The right aster and its partly formed central spindle is associated with the lower nucleus, while the central spindle of the left aster penetrates the upper nucleus. The direction of the two half-spindles emphasizes their independence. Similar conditions are further illustrated in Figure 89, where the section has been made in such a direction that the two pronuclei do not cover each other as in the previous cases, and therefore the relation of the asters to the spindle halves is more patent. The peripheral ends of the spindle fibres which are in relation with the larger (right) pronucleus are all bent, a condition observed in this one instance alone.

The centre of each aster is occupied in all of these cases by a sharply defined centrosome, which is enveloped in a layer of sphere-substance that later becomes differentiated into cortical and medullary layers.

That the cleavage centrosomes arise one in connection with each of the pronuclei, is further indicated by the fact that in more than one hundred eggs in which only one pronucleus occurred in the section, there was never found a trace of more than one aster primarily associated with it. It seems improbable that this can be due in so many cases solely to the accident of cutting. This observation has been verified by a careful examination of a number of whole eggs; the earliest stages observed showed two asters in the cytoplasm, lying at some distance from the nuclear membrane, and no evidence was found to indicate that these asters were derived from pre-existing asters.

As the pronuclei are invaded by the central spindle, their walls break down and the contents are differentiated into two substances: first, the solid, or partly solid, chromatin masses that are to become the chromosomes (Plate **13**, Fig. 93; Plate **12**, Figs. 86, 81, 82); secondly, a rather coarsely granular substance, which takes a heavy stain in iron hæmatoxylin. The latter is more abundant when the nuclei first break down (Fig. 93), but gradually becomes less and less until it is indistinguishable in the cytoplasm (Fig. 82). During this period there has been an active metabolism in the cell, as is shown by the condition of the cytoplasm in the region that marks the transition from it to the deutoplasm. This condition is probably due to the breaking down of the yolk spheres into coarse granules, which take a basic stain and are definitely limited to this region of the egg. Opposite the equatorial plane of the spindle these granules extend further into the cytoplasm than in other regions (Figs. 81, 93). When the egg reaches the metaphase, this condition disappears. An examination of the sections adjacent to those figured indicates that the irregular area on the polar (or formative) side of the young cleavage spindle is not a part of the cytoplasm in which the active metabolic changes are taking place, but is to be looked on as having a nuclear origin. An examination of the female pronucleus in Figure 51 (Plate **8**) shows that there is a great deal more chromatin in the nucleus than is necessary to form the chromosomes. The ring-shaped chromosomes, shown in Figures 87 (Plate **12**) and 90 (Plate **13**), were derived from solid masses of chromatin, which during this transformation changed in its reaction to stains. They cannot be individually followed to the chromosomes of the cleavage figure. The division of the chromosomes is transverse (Plate **12**, Fig. 82), and they migrate to the poles of the spindle as in maturation. In the late anaphase the chromosomes become vesicular and fuse (Plate **13**, Figs. 92, 91, 97), to form subsequently the quiescent nucleus, as in the anaphase of the second maturation.

The thickening of the interzonal filaments begins to appear in the region of the equatorial plane during the anaphase; this differentiation arises not only on the interzonal filaments, but also on some of the rays farther from the axis of the figure (Figs. 91, 92). As the cleavage furrow deepens, it carries with it the central part of the interzonal filaments and unites the thickenings on them into a single body, the "Zwischenkörper" (Fig. 97).

The centrosome is a solid body until after the metaphase (Plate **13**, Fig. 93; Plate **12**, Fig. 82). In favorable sections the two regions, centriole and centroplasm, can be distinguished in the anaphase, but the centrosome in cleavage is so small that it is difficult to see precisely how its differentiation takes place. Around the centrosome are the cortical and medullary layers of the sphere, the medullary layer increasing in extent during the late anaphase (Fig. 92). As the vesicular chromosomes fuse to form the quiescent nucleus, the medullary and cortical layers become diffused in the cytoplasm (Fig. 91). The centrosome in the late anaphase is somewhat irregular in outline (Fig. 92). The centriole can be seen in one of the centrosomes of this stage, but not in the other, and during the next period the rays still converge toward a body which takes a slightly heavier stain than do the microsomes in the cytoplasm, but no centrosomal differentiation could be discovered in it (Fig. 91). Apparently the centrosomes of the first cleavage spindle do not divide in preparation for the next cleavage, but gradually disappear, until there is no evidence of a centrosome in the egg (Fig. 97). Both centrosome and sphere become indistinguishable, as in Limax agrestis (Byrnes, '99).

The appearance of the nucleus in the telophase of the first cleavage is represented in Figure 95, which was constructed by superposing the outlines of the pronuclei in three successive sections.

In the prophase of the second cleavage the chromatin passes through a metamorphosis similar to that of the prophase of the first cleavage. Asters arise independently in the cytoplasm ; they may lie on diametrically opposite sides of the nucleus, or several degrees away from such a diameter (Fig. 96). A large amount of material was examined in the stages that intervene between the anaphase of first cleavage and the prophase of second cleavage, but in no instance was it possible to trace any connection between the asters in the prophase of the second cleavage and the centrosome of the previous stage. The changes in the second cleavage are a repetition of those occurring in the first. The centrosomes entirely disappear, and therefore their fate cannot be followed.

The prophase of the third cleavage is shown in Plate 13, Figure 98. The section passes through two of the four cells and through three of the new asters. These new asters have as yet no connection with each other. It looks as though the nuclear wall in the cell showing two asters were drawn out in the direction of the aster which is farthest from the animal pole. The cytoplasm in the vicinity of the deutoplasm stains intensely, indicating a stage of great metabolic activity. This phenomenon occurs at a corresponding period in the second cleavage (Fig. 96), but the section does not pass through the part of the egg which shows this staining property.

The history of the nuclei and centrosomes during subsequent cleavages is the same as that during the three described, the cleavage centrosomes arising anew at each generation.

Polyspermy. Occasionally two or three sperm heads are found in an egg; each of these usually moves toward the animal pole until the female pronucleus is reached ; then there arises a tripolar or a tetrapolar figure. When these abnormal cleavage figures arise, segmentation is rarely completed, the egg remaining in a partly formed two-cell stage. In some capsules more than half the eggs were thus abnormal. In eggs in which the abnormal figures occur, I have found that the number of asters which unite to form these multipolar figures does not indicate a doubling of each extra male pronucleus, but that a single aster arises for each. In Haminea, then, a tripolar figure indicates that there were two male pronuclei; a tetrapolar figure, that there were three male pronuclei in the egg. This fact furnishes additional evidence in favor of the view that one of the cleavage centrosomes is associated with each pronucleus.

(c) *Discussion of Results and Literature.*

The only account of the development of Haminea that has appeared is the preliminary report by Crampton (**'97**, p. 63), which I quote in full: "The observations were made upon the eggs of a species of Doris, collected last summer on the Pacific Coast by Mr. Calkins, and upon a species of Bulla [Haminea] which deposited eggs at Woods Hole during the months of August and September. The results may best be summarized by stating that a complete confirmation was obtained of the account of fertilization given by Wilson and Mathews, Boveri, Hill for sea urchins, Meade on Chætopterus, Kostanecki and Wierzejski upon Physa, etc. The sperm nucleus is preceded by the divided centrosome, an aster, however, not being found till the union of the germ-nuclei. The first polar spindle has at each pole a double centrosome, the second maturation spindle but one. These are of great size, however, and the one remaining in the egg finally disintegrates, the centrosome of the first cleavage spindle being derived from the sperm. The germ-nuclei do not fuse, but lie very close to one another, in contact."

It is generally conceded now that it is unsafe to apply the term "centrosome" to a body that shows no evidence of association with astral rays. The fact that no rays were found by Crampton in connection with the sperm centrosome renders his conclusions open to serious question. If a centrosome does not precede the sperm head in its migration in the egg, — and I believe that it does not, — then my observations in regard to these changes agree with those made by Crampton.

We may now inquire into the significance of the bodies in Haminea (Bulla) which I have described as accessory asters.[1] Similar conditions have been observed in other Mollusca, and this same terminology is probably applicable to the structures designated by some writers as sperm asters.

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[1] For a full description of the general subject of accessory asters, see Wilson (**:01**^{a}, pp. 560 ff., 580 ff.).

Upon this question Lillie (:**01**, pp. 244, 245) says: "Shortly after the metaphase of the second maturation spindle an accessory aster is formed in the egg. It usually arises quite near the center of the egg and bears no fixed relation either to the maturation spindle or the sperm-nucleus; the center of the aster is occupied by an exceedingly minute centrosome. The latter divides and a small amphiaster is formed which entirely disappears. . . . What is the significance of the accessory aster? Is it simply due to a renewal of activity of the sperm-centrosomes, which disappeared a cell generation before, or is it an entirely new formation? I do not believe that it has anything to do with the original sperm-asters for several reasons: in the first place, there are two sperm-centrosomes, and the accessory aster is at first single and later divides; secondly, the usual place of origin of the accessory aster does not correspond with the usual place of disappearance of the sperm-amphiaster; and, finally, in the egg of Crepidula (Conklin, '**98**[a]) there are two or three accessory asters formed at about this time, while the sperm-aster is perfectly distinct in connection with the sperm-head."

In a former paper (Smallwood, :**01**, p. 150) I described the conditions represented in text-figure 5 of that article as belonging to the process of fertilization, stating that there was a sperm aster. This egg has been examined again (Plate **9**, Fig. 59), and the aster found to have no relation to fertilization; this was the only case in which rays were found associated with the sperm head. I now believe that the asters found in Haminea are to be interpreted as accessory or secondary asters, that they are new, short-lived formations, which do not undergo further development, and bear no relation to the origin of the cleavage centrosomes.

Byrnes ('**99**) found only two definite asters in the egg of Limax agrestis during the migration of the sperm head. These asters are termed sperm asters; but in general appearance, time of occurrence, and position, they correspond to the asters termed accessory by Conklin and Lillie. It seems to me that this is the correct interpretation for these structures in Limax, and that the derivation of the cleavage asters from the sperm asters in Limax is highly improbable.

The literature of fertilization has been so recently summarized by Coe ('**99**), Wilson (:**00**), and Lillie (:**01**) that a review of it need not be given here.

That the cleavage centrosomes arise *de novo* from the cytoplasm of the egg has been maintained by the following writers: Foot ('97) in Allolobophora fœtida, Child ('98) in Arenicola, Lillie ('97, :01) in Unio, Foot and Strobell (:01, p. 606) in Allolobophora fœtida, and Smallwood (:01) in Bulla. Conklin (:01) has shown for Crepidula that the cleavage centrosomes arise, one from the male sphere-substance, the other from the female sphere-substance; while Wilson (:01^b) has proved conclusively that centrosomes may arise in connection with the female pronucleus. In describing the effects of ether on Arbacia eggs he says (:01^b, p. 360) in regard to the union of the male with the female pronucleus, "As a rule the nuclei remain distinct, separately undergoing a highly interesting series of changes. The most striking fact is that while the sperm-aster often gives rise to a perfect and symmetrical bipolar division-figure, the egg nucleus, in a great number of cases, produces a monaster which seems at first incapable of resolving itself into a bipolar figure." He adds (p. 362), "Although the egg-monaster does not at first divide, it may do so in later stages."

The relation of the two pronuclei to the cleavage asters at their first appearance indicates a great want of agreement among different animals, although the conditions remain constant for each species. In Pleurophyllidia (MacFarland, '97) the cleavage asters arise in almost any relation to each other and to the two pronuclei; while in Toxopneustes (Wilson, '95), in Chætopterus (Mead, '98), in Cerebratulus (Coe, '99), and other invertebrates the new cleavage asters bear a very definite relation to each other and to the two pronuclei. Between these two extremes are found intermediate conditions in which the new cleavage asters have no fixed relation to each other, but a very definite one to the pronuclei, inasmuch as one of them is associated with each pronucleus. Such an intermediate condition has been observed by Lillie (:01) in Unio, by Conklin (:01) in Crepidula, and by Smallwood (:01) in Bulla (Haminea).

Linville (:00) figures for Limnæa elodes and Limax maximus conditions which show that one of the new cleavage asters may be found associated with each pronucleus. Linville believes this to represent a "temporary and incidental" state. In two of the three eggs reproduced the asters are the youngest ones represented by him. It is entirely conceivable that the asters may become united to each other, and the association with each pronucleus may be a temporary one; but it does not seem to me to be incidental in Haminea, because, from the conditions in a large number of eggs examined, it is clearly unusual for both the new cleavage asters to be exclusively associated with either of the two pronuclei. Moreover, Linville apparently draws his conclusions from the later rather than the very earliest stages. It is probable that more extended observations, and especially the study of stages earlier than those reproduced by him, would reveal a more definite relation between the earliest traces of the cleavage asters and the pronuclei. Evidently, then, the more recent investigations on Mollusca indicate that each pronucleus is associated in origin with one and only one of the cleavage asters.

5. SUMMARY.

First Maturation. Ovocyte. The cytoplasm of the young ovocyte is of homogeneous appearance. When deutoplasm begins to form in it, vacuoles appear in the nucleolus. These increase in number and size; by their confluence the nucleolus comes to contain a single large vacuole. The nucleus contains both basichromatin and oxychromatin granules.

Centrosome. The centrosome in the earliest condition observed is composed of five bodies; these unite into one before the egg is laid.

The cortical layer of the "sphere" enveloping the centrosome is finely granular, free from yolk spheres, and destitute of the reticulate structure of the cytoplasm.

The centrosome, at first homogeneous, becomes differentiated into centriole and centroplasm. The centroplasm is, by definition, never traversed by astral rays. At the same time the sphere-substance is differentiated into a cortical and a medullary layer. During the formation of the centroplasm its periphery has an irregular outline, which continues until the centrioles divide.

Chromosomes. The chromosomes first appear in the form of deposits on the walls of chromosomal vesicles which are derived chiefly from the linin threads of the germinative vesicle. When the chromosomes have attained considerable size, the walls of the chromosomal vesicles disappear. The spireme of the germinative vesicle does not give rise to the chromosomes by either segmentation or splitting of its substance; but there intervenes between the two a stage in which the chromatic substance, reduced to a minimum, can be detected only as a partial investment of the linin network of the disintegrating germinative vesicle The individuality of the chromosomes cannot, therefore, be demonstrated in Haminea.

Second Maturation. The eggs of Haminea form the second maturation figure by either one or the other of two somewhat different, but apparently normal, processes. Only one of the processes occurs in eggs laid by a given individual. Of these two processes, the one which I have called the shorter or direct is the simpler and more like that known to exist in most other animals. In it the chromosomes neither lose their individuality nor pass through the network stage characteristic of the so-called quiescent nucleus. They are never found far removed from the animal pole of the egg. In the longer or indirect process the young achromatic figure is formed at a distance from the animal pole, and with the immediately surrounding cytoplasm moves peripherally. The chromosomes of the inner half of the first maturation figure in this process pass into the state known as the quiescent nucleus, characterized by the presence of a reticulum. While in this stage they divide in preparation for the formation of the second polar cell.

Some of the chromosomes become vesicular (Plate **6**, Fig. 32 ; Plate **7**, Figs. 40, 43) in both processes, but this condition is not assumed at any definite time.

The central spindle arises at the time of the formation and moving apart of the two new centrioles ; later it is dissolved. The centrosomes move far apart, and a new spindle is formed between them. The central spindle is an outgrowth from the chromosomes ; but the mantle fibres are formed from the cytoplasm. During the separation of the centrosomes there is a marked change in the distribution of the cytoplasm in the egg ; this indicates that the total contents of the ovum are affected by the movement of the centrosomes, or that both owe their activity to a common cause. The two processes, the direct and the indirect, are practically identical from the time of the metaphase onward.

During the second maturation phenomena the centrosomes pass through a metamorphosis similar to that which occurs during the first maturation process ; but in the indirect process there is a noticeable lack of synchronism in the development of the two centrosomes in relation to each other and to the metamorphosis of the chromosomes.

The centrosome at the deep end of the second maturation spindle disappears at the close of maturation. The corresponding chromosomes pass into a vesicular state and finally fuse to form the female pronucleus. The sphere-substance around the female pronucleus also becomes unrecognizable before cleavage begins.

In the first maturation the chromosomes divide transversely, but in the second maturation it is difficult to ascertain whether the division is transverse or longitudinal. A quantitative, but not a numerical, reduction is accomplished by each of these two divisions. Since the chromosomes of the germinative vesicle probably lose their individuality during the formation of the first maturation spindle in the ovotestis, qualitative reduction cannot be demonstrated in Haminea.

Fertilization. The sperm head may penetrate the ovum at any point of its surface. A middle piece could not be distinguished. The sperm head is not accompanied by a sperm aster, nor is there any sphere-substance associated with the sperm head.

Accessory asters appear in the anaphase of the second maturation; these are temporary and independent of the sperm head.

Cleavage. The chromatin in both male and female pronuclei passes through an elaborate metamorphosis before the cleavage asters arise. Of the two cleavage asters one is associated in origin with each pronucleus.

The centrosome at the close of each cleavage becomes indistinguishable from the microsomes in the cytoplasm, and it is therefore impossible to affirm that it divides in preparation for the following cleavage.

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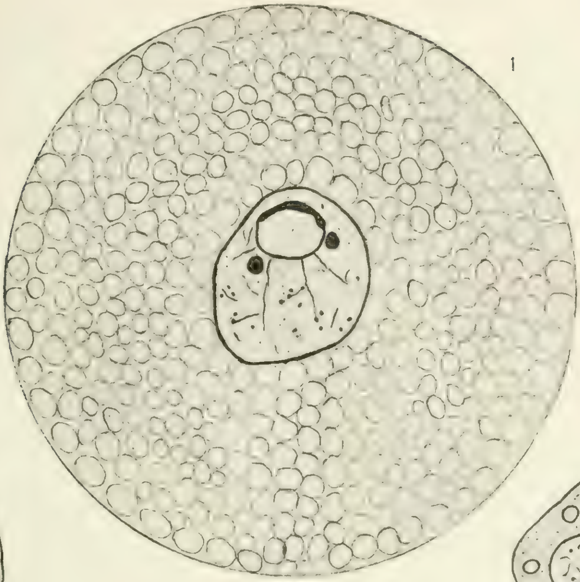
PLATE 1.

Fig. 1. Full-grown ovarian egg from ovotestis. The large quantity of deutoplasm, represented diagrammatically, obscures the structure of the cytoplasm. There are two plasmosomes in the nucleus. Nucleolus contains a large vacuole, which has taken the place of most of the chromatin. Iron hæmatoxylin. × 1224.

Fig. 2. Young ovocyte from ovotestis. The cytoplasm is granular, but does not contain yolk spheres. Nucleolus solid. Iron hæmatoxylin. × 1224.

Fig. 3. Ovocyte from ovotestis. A few deutoplasmic spheres in the cytoplasm. Four vacuoles in nucleolus. Iron hæmatoxylin. × 1224.

Fig. 4. Egg taken from the hermaphroditic duct. The beginning of the first maturation figure. Centrosome composed of five solid bodies surrounded by a cortical layer of sphere-substance. The central spindle passes through the germinative vesicle. Two chromosome vesicles have partly formed. Iron hæmatoxylin. × 1680.



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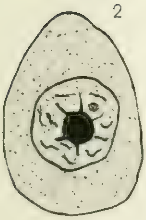

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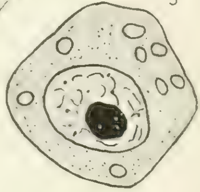

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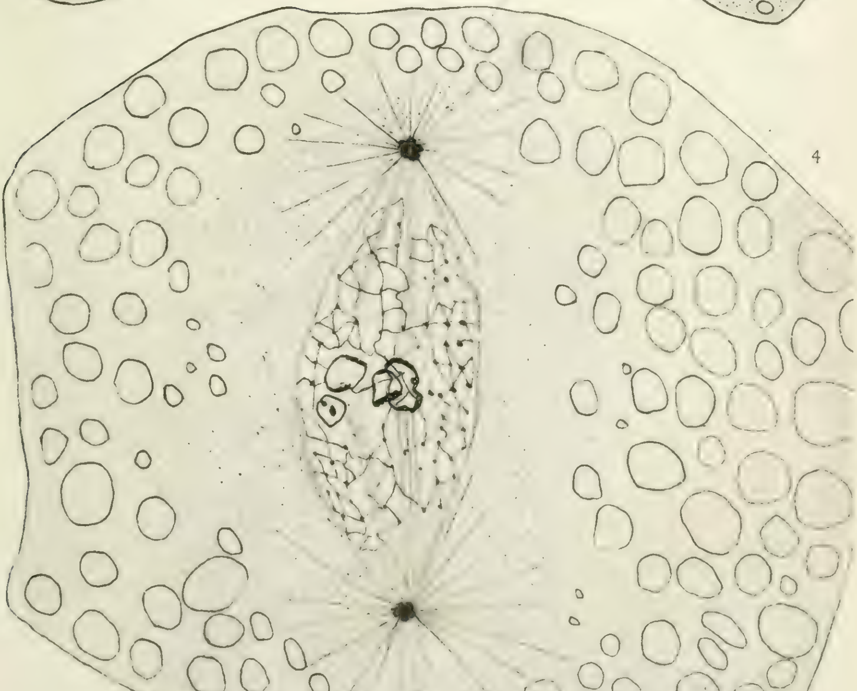

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PLATE 2.

Fig. 5. Polar view of the centrosome of an egg taken from the hermaphroditic duct. Iron hæmatoxylin. × 1680.

Fig. 6. Section of centrosome in a plane oblique to the axis of the first maturation spindle, from an egg taken from the ovotestis. Iron hæmatoxylin. × 1680.

Fig. 7. Nucleus of growing ovocyte from the ovotestis. Vacuoles in the nucleolus displacing the chromatin. Iron hæmatoxylin. × 1224.

Fig. 8. Nucleus of growing ovocyte from the ovotestis. The contents of the nucleus is granular. Delafield's hæmatoxylin and eosin. × 1224.

Figs. 9–12. Four successive stages in the metamorphosis of the germinative vesicle. The centrosome becomes smaller, and the five bodies more or less completely fuse with one another. The central spindle is present, showing the formation of the chromosome vesicles and the accumulation in them of chromatin. Iron hæmatoxylin and Bordeaux red. × 1680.

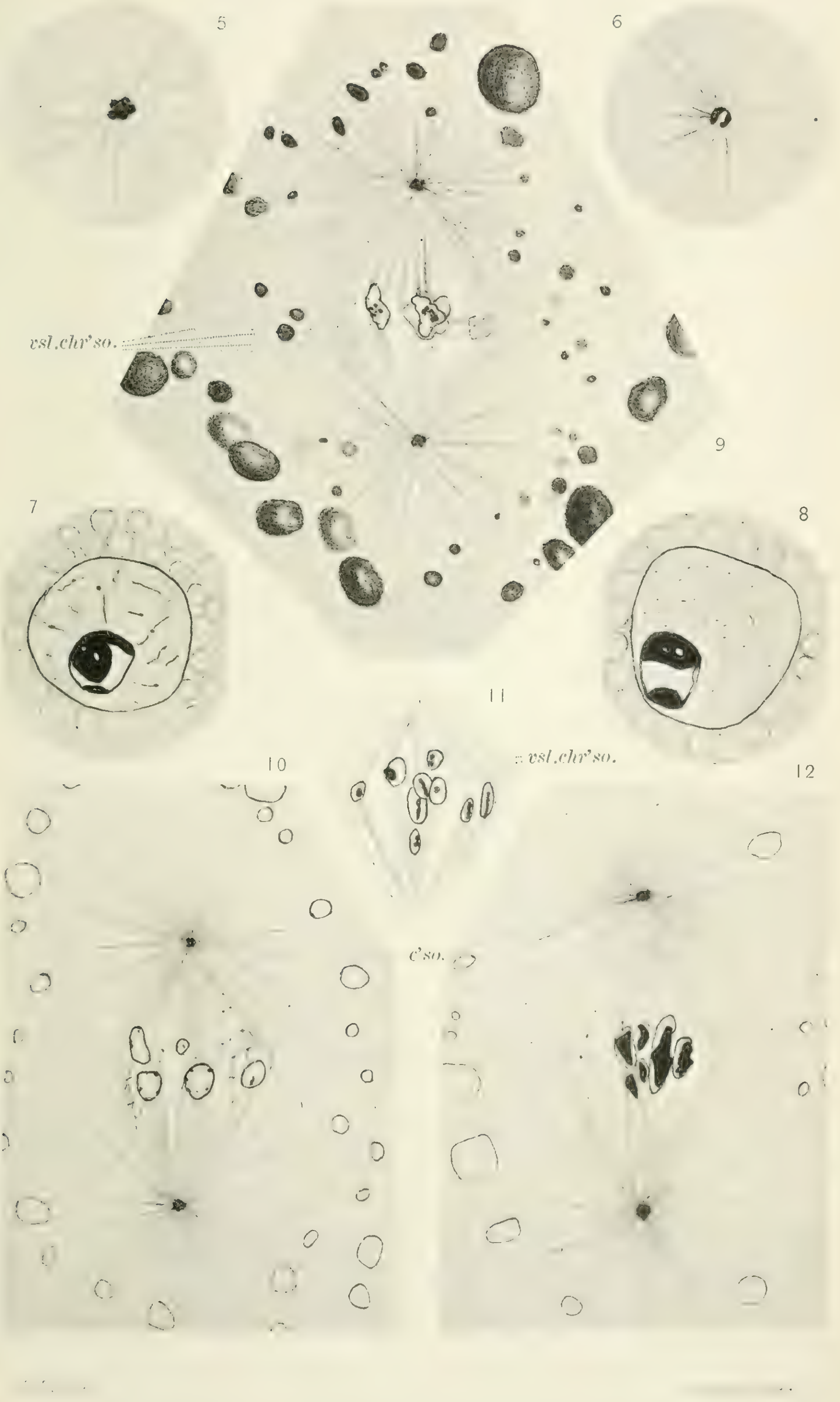
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PLATE 3.

Fig. 13. Section of an egg parallel to axis of first maturation spindle. The maturation figure occupies the centre of the egg and is surrounded by cytoplasm which is mostly free from yolk spheres. The central spindle has disappeared. The chromatin in each of the chromosome vesicles consists of three masses arranged in a linear series or a three-lobed mass. The centrosome is a single solid body. Egg from ovotestis. Iron hæmatoxylin and Bordeaux red. × 1680.

Figs. 14, 15. Sections through the germinative vesicle perpendicular to the axis of the forming spindle in the region of its equatorial plane. Chromosome vesicles are definite in outline. The chromatin is located on one side or projects out into the vesicle. Linin threads disappearing. Egg taken from the ovotestis. Iron hæmatoxylin and Bordeaux red. × 1680.

Fig. 16. Portion of a section from an egg taken from the ovotestis. The chromosome vesicles enclosing the chromosomes have disappeared. Chromosomes three-lobed. Centrosome differentiated into thin centroplasm layer and a centriole. Iron hæmatoxylin and Bordeaux red. × 1680.

Fig. 17. Section of the germinative vesicle perpendicular to the spindle axis in region of equatorial plane. The chromosome vesicles are connected to one another by linin threads. The cytoplasm is somewhat condensed and finely granular immediately around the germinative vesicle. Taken from the ovotestis. Iron hæmatoxylin and Bordeaux red. × 1680.

Fig. 18. Portion of section of an egg, showing the primary astral rays extending to the periphery of the egg. There are on them small bodies. The secondary rays are much shorter and finer than the primary ones. Delafield's hæmatoxylin and picric acid. × 1680.

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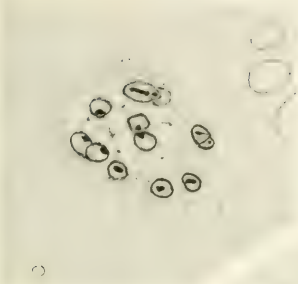

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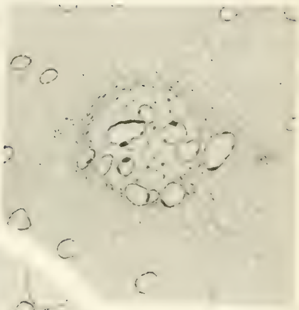

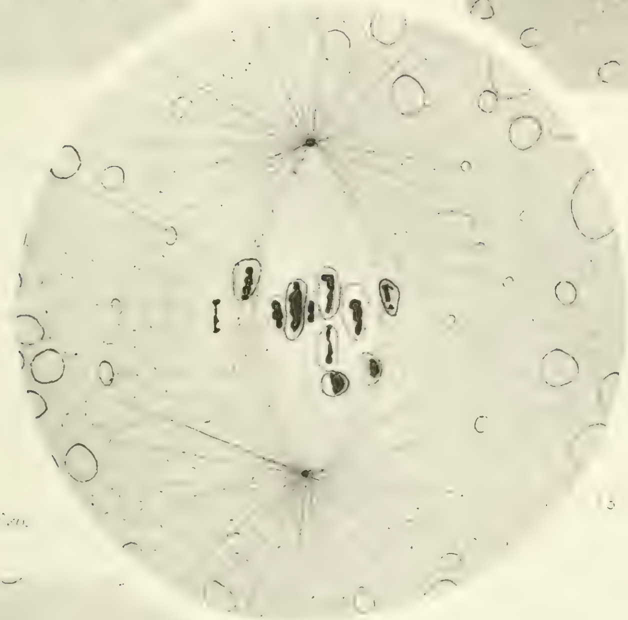
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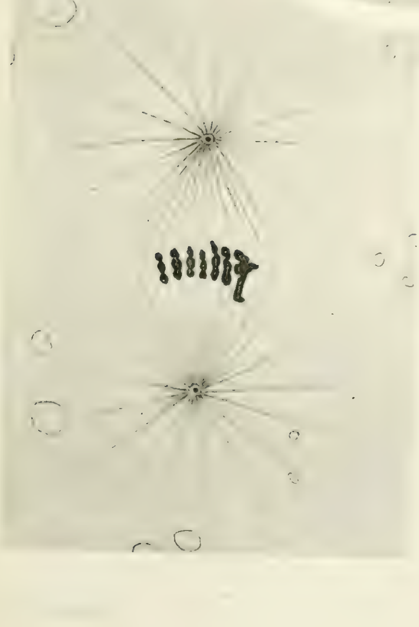

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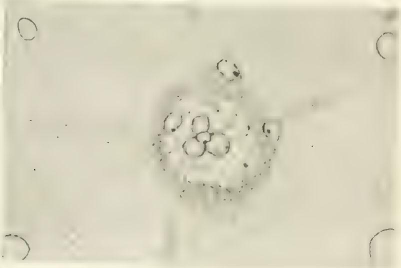

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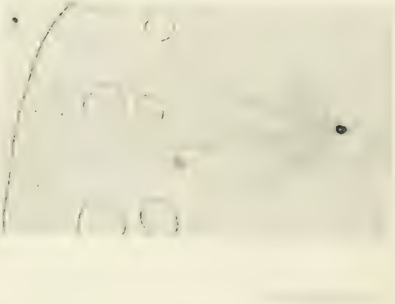
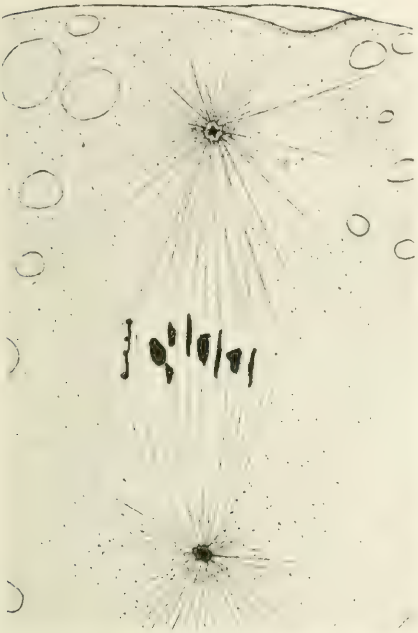


PLATE 4.

Figs. 19–22. Metaphase of first maturation spindle. Chromosomes mostly rodlike. Centrosome differentiated into centriole and centroplasm. Sphere-substance composed of a medullary and a cortical layer. Iron hæmatoxylin and Bordeaux red. × 1920.

Fig. 23. A section of the centrosome. Centriole large and irregular in outline. Centroplasm crenate, projecting out in the direction of some of the astral rays. Medullary layer takes a faint plasma stain. Iron hæmatoxylin. × 1920.

Fig. 24. A cross-section of the first maturation spindle in the metaphase. Sixteen chromosomes. Fibres of central spindle show as fine dots between the chromosomes. Iron hæmatoxylin. × 1224.



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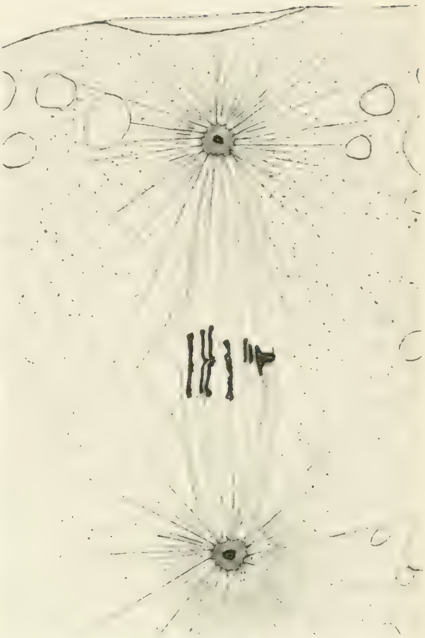

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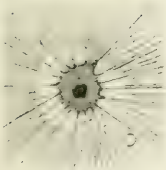

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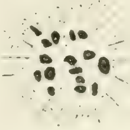

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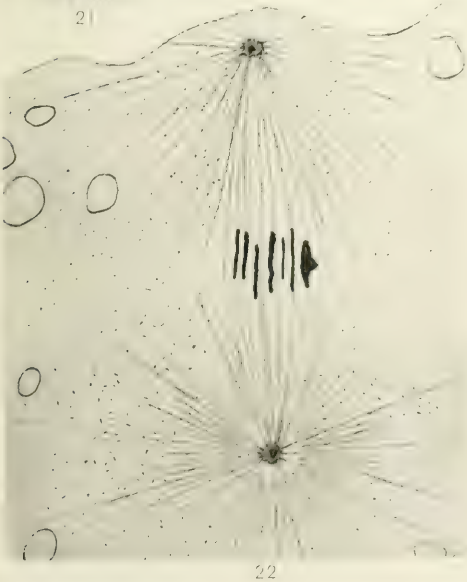

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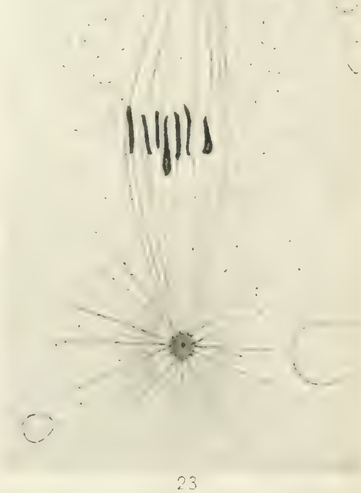

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PLATE 5.

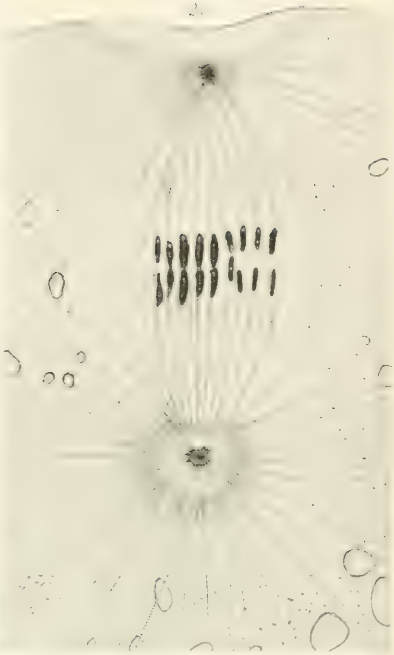
Fig. 25. Metaphase of first maturation. The centriole in the deep centrosome is represented by several small granules. The chromosomes are still in the tripartite condition. Iron hæmatoxylin and Bordeaux red. × 1920.

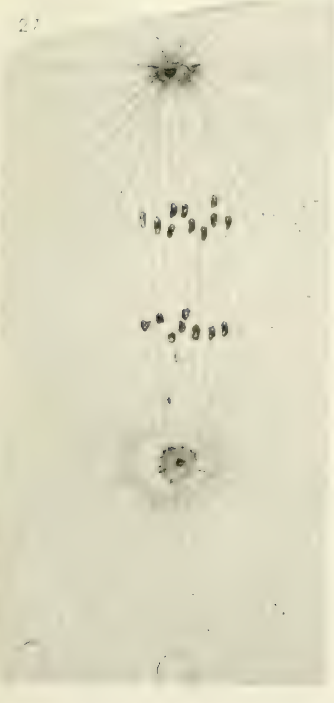
Figs. 26, 27. Beginning of anaphase of first maturation. The chromosomes are moving toward the poles of the spindle. Iron hæmatoxylin. × 1920.

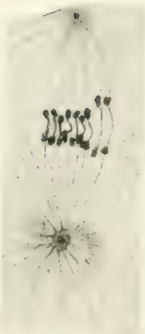
Fig. 27*a*. The centriole in the dumb-bell stage. Taken from an egg in the anaphase. Iron hæmatoxylin. × 1920.

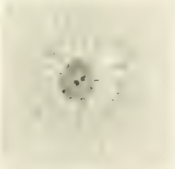
Fig. 28. Late anaphase of first maturation. The centriole has broken up into a number of granules. In the outer centrosome no differentiation (centriole) is found in the centroplasm. Iron hæmatoxylin and Bordeaux red. × 1920.

Fig. 29. Chromosomes are dividing. A deeply staining chromatin thread exists between the components of each pair. Iron hæmatoxylin. × 1224.









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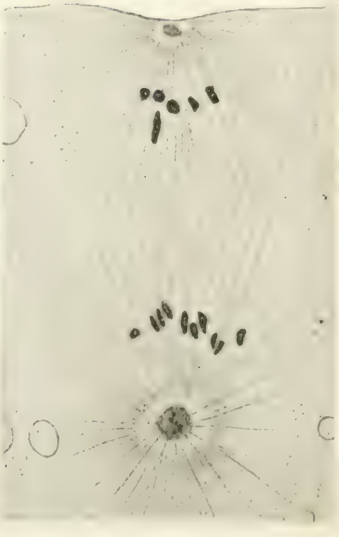


PLATE 6.

All figures except 32*a* magnified 1224 diameters.

Fig. 30. Anaphase first maturation. The centrosome of the forming polar cell has disappeared. Two centrioles in deep centrosome. Chromosomes arranged in the form of a ring. Iron hæmatoxylin.

Figs. 31, 31*a*. Two successive sections showing prophase of second maturation. Figure 31*a* represents the deeper section. Centrosomes faint. Chromosomes at some distance from axis of nascent spindle. Brazilin.

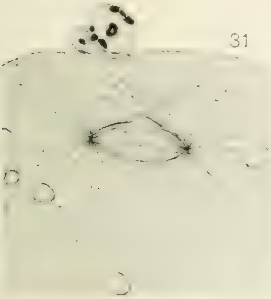
Fig. 32. Second maturation spindle assuming a radial position. Chromosomes vesicular. Polar cell in the anaphase of division. Iron hæmatoxylin and Bordeaux red.

Fig. 32*a*. Polar cell of Figure 32 more highly enlarged. × 2448.

Figs. 34, 36. The maturation spindle occupies a radial or nearly radial position. The centrosome is differentiated into centriole and centroplasm. The chromosomes (Fig. 36) are dividing. Iron hæmatoxylin and Bordeaux red.

Fig. 35. The second maturation spindle lies at right angles to the radius of the egg. Centrosomes are elongated. Brazilin.

Fig. 37. First polar cell abnormally large. There is an accessory chromosome in the egg and another in the polar cell. Iron hæmatoxylin.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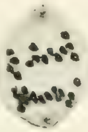
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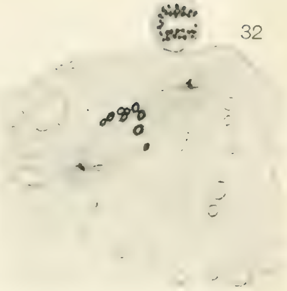
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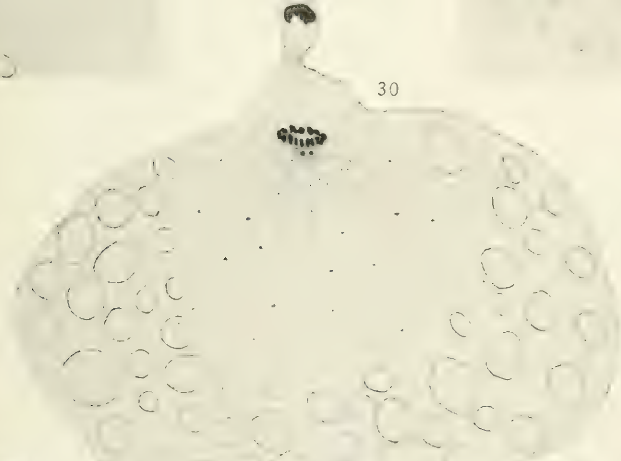
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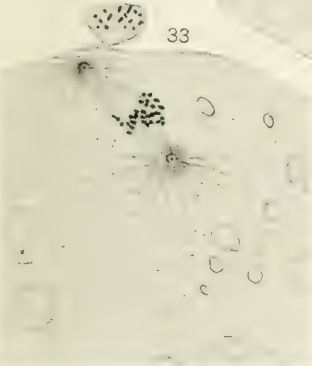
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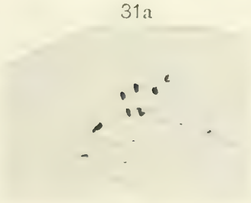
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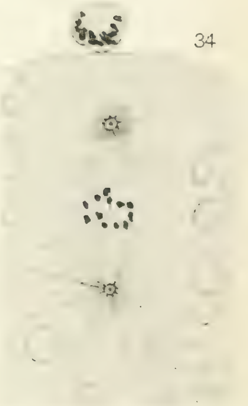
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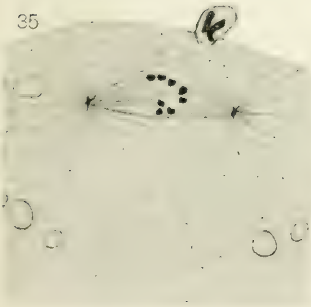
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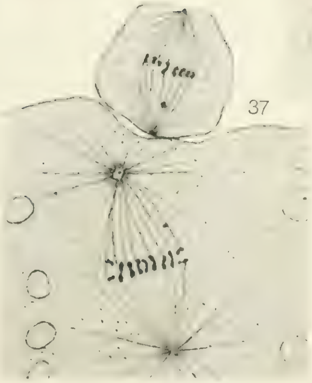
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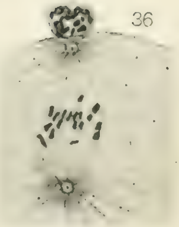
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PLATE 7.

All figures magnified 1224 diameters.

Figs. 38, 39. Radial sections through the animal pole of the egg. First maturation spindle in anaphase, the chromosomes having divided. The egg chromosomes in Figure 38 are surrounded by a nuclear membrane, with which they are connected by linin fibres. At both poles of the spindle, the centrosome contains two centrioles joined by a central spindle. Iron hæmatoxylin and Bordeaux red.

Fig. 40. First polar cell formed. The egg chromosomes have become vesicular and are joined to the nuclear membrane by linin fibres. In both Figures 39 and 40, the bounding wall of the centroplasm of the egg centrosome is no longer continuous, being broken at several points; beginning of its disintegration. The centrioles are connected by a central spindle. Iron hæmatoxylin and Bordeaux red.

Fig. 41. Egg chromosomes separated into two clusters, each with a nuclear membrane and linin fibres. A thin-walled vesicle without chromosomes lies near the periphery of the egg, almost in contact with the parent centrosome, which has increased in size, and the degenerating wall of which has become broken and less distinct. The new centrosomes have accumulated around themselves a cortical layer of sphere-substance, in which new short radiations are established; spindle much elongated. Old radiations, centering in the enlarged parent centrosome, have not entirely disappeared. Iron hæmatoxylin and Bordeaux red.

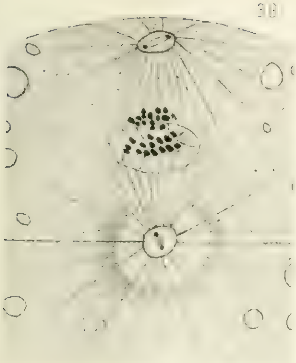
Fig. 42. Achromatic figure of second maturation spindle larger, although the first polar spindle has advanced only to the close of the metaphase. The wall of the parent centrosome, though conspicuous, is interrupted; the rays of the new centrosomes more prominent and longer than in Figure 41; those of the parent centrosome still conspicuous and extensive, the contrast between the cortical and medullary portions of the old sphere-substance being well marked. The accessory chromosome of the first spindle is partly enveloped in a nuclear membrane. Iron hæmatoxylin and Bordeaux red.

Fig. 43. First polar cell already formed. Chromosomes vesicular, though not yet surrounded by a nuclear membrane, and some of them connected with linin fibres. The new centrosomes of the second spindle somewhat larger than in Figure 42, and a greater accumulation of new sphere-substance about them. Iron hæmatoxylin.

Figs. 44, 45. Achromatic figure enlarged and moving into a radial position at the animal pole. The boundary of the old centrosome much interrupted in Figure 44, and wholly wanting in Figure 45. In Figure 44, the central spindle is narrow and well defined; in Figure 45, it is more swollen and spindle-shaped. In this figure medullary and cortical layers of the old sphere-substance have become limited to the deep pole of the spindle. Chromosomes still surrounded by a nuclear membrane, to which they are connected by linin fibres. Iron hæmatoxylin and Bordeaux red.

Fig. 46. The central spindle has nearly disappeared; centrosomes farther apart; granules in the cytoplasm about the asters arranged into rows preparatory to the formation of additional rays. Iron hæmatoxylin and Bordeaux red.

Figs. 47–49. Three successive stages. The asters have moved much farther apart. New cytoplasmic rays forming between asters and nucleus. The deep centrosome has become differentiated into centriole and centroplasm. In Figure 47 the sphere-substance around the deep centrosome is composed of a medullary and a cortical layer, and both nuclear membrane and linin fibres are conspicuous; in the following stages (Figs. 48, 49) both become less so and finally disappear. The chromosomes in Figures 48, 49, have divided and from them have grown out fine (spindle) fibres.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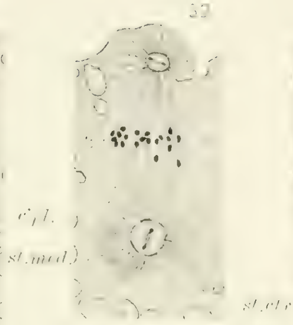
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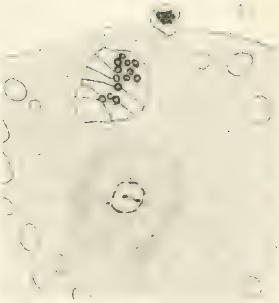
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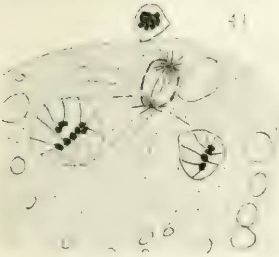
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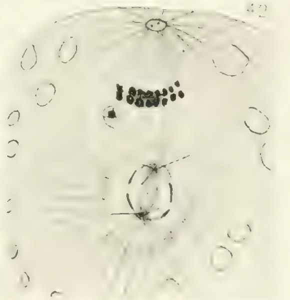
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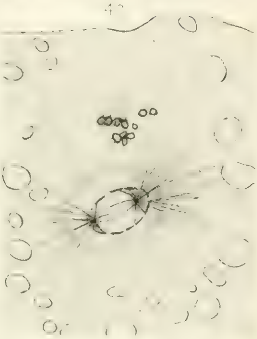
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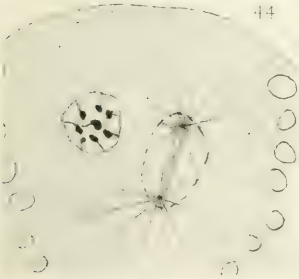
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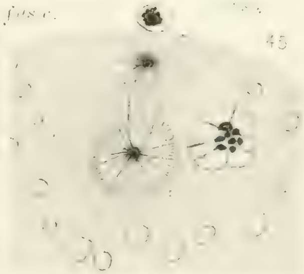
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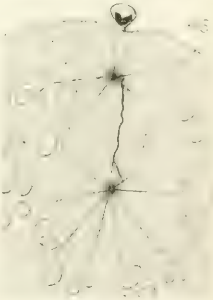
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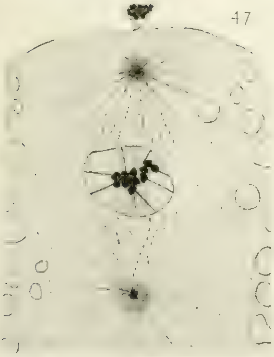
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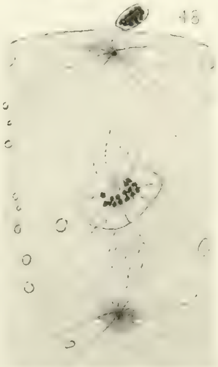
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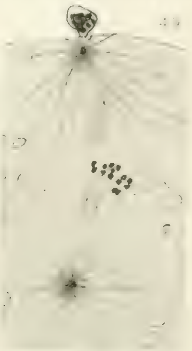
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PLATE 8.

Fig. 50. Late anaphase of second maturation. The whole centrosome is very faint. Iron hæmatoxylin and Bordeaux red. × 1224.

Fig. 51. The chromatin of the female pronucleus is in the sponge-work stage. Iron hæmatoxylin. × 1224.

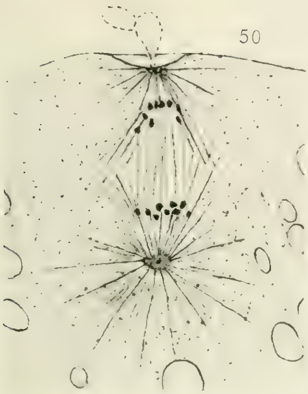
Fig. 52. Anaphase of second maturation. Minute centriole present in flattened centrosome. Iron hæmatoxylin and Bordeaux red. × 1224.

Fig. 53. The male pronucleus lies near the centre of the egg. Interzonal filaments still connect the second polar cell with the female pronucleus. Division of the first polar cell nearly completed, and exhibiting a small midbody. Iron hæmatoxylin and Bordeaux red. × 1224.

Fig. 54. Second maturation figure. The centrosome composed of centriole and centroplasm. Sphere-substance differentiated into medullary and cortical layers. Spindle very long, but incomplete. Fine fibres growing out from chromosomes toward centrosomes. Brazilin. × 1224.

Fig. 55. Second maturation. Spindle elongated still more and nearly complete. Outer centrosome still homogeneous. Inner one differentiated into centriole and centroplasm. Brazilin. × 1224.

Figs. 56, 57. The sperm head at the beginning of its migration. Vacuolation has not yet taken place in it. Delafield's hæmatoxylin and picric acid. × 1680.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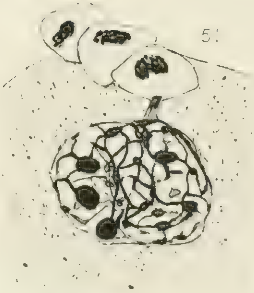
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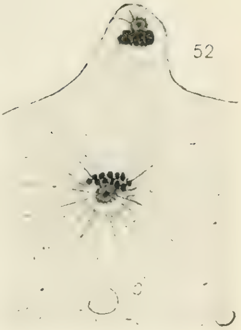
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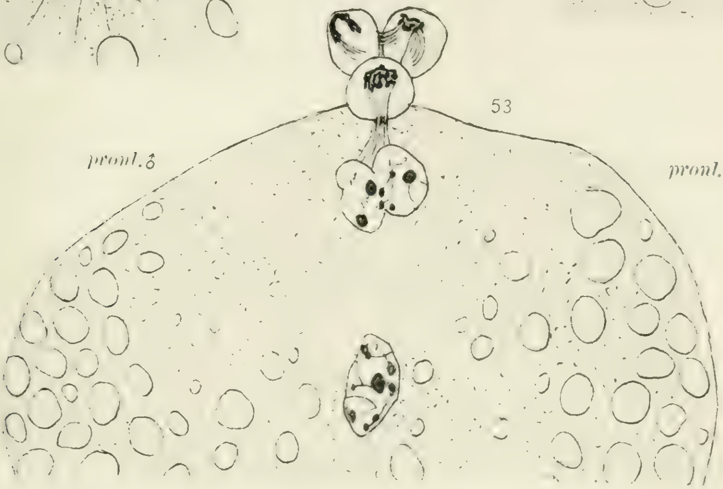
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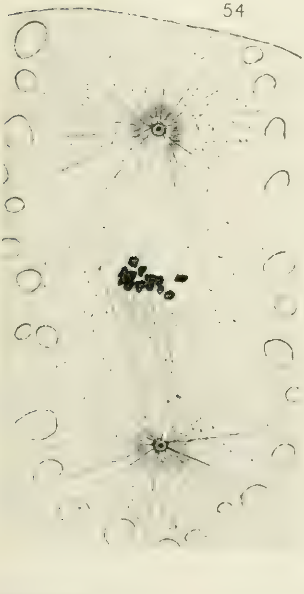
pronl. ♂
pronl. ♀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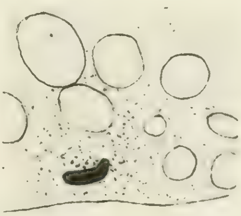
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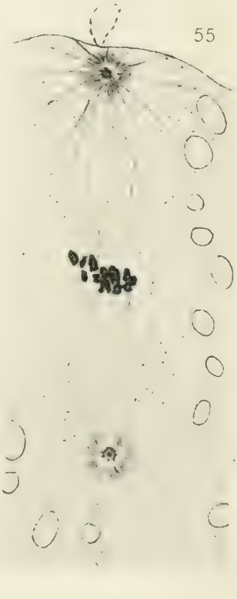
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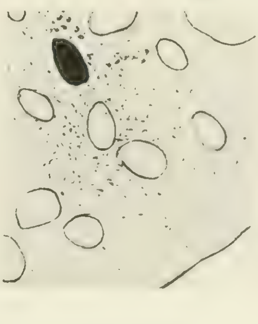
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PLATE 9.

Fig. 58. The young achromatic figure still partly surrounded by the bounding wall of the parent centroplasm. Old astral rays disappearing; new astral rays few, but prominent. Boundary between cortical and medullary portions of sphere-substance still visible on one side of the figure. Iron hæmatoxylin. × 1224.

Fig. 59. The deep (egg) chromosomes have passed into the telophase, although the second polar cell has not yet been formed. Brazilin. × 1224.

Figs. 60–64. Show the approach of the spermatozoön and migration of the sperm head after penetrating the egg. Sperm nucleus becomes vesicular. Delafield's hæmatoxylin and picric acid. × 1680.

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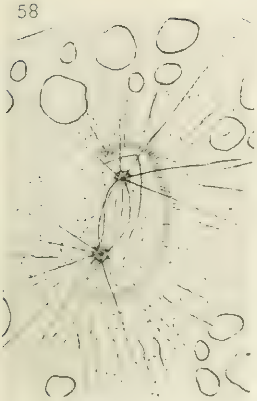

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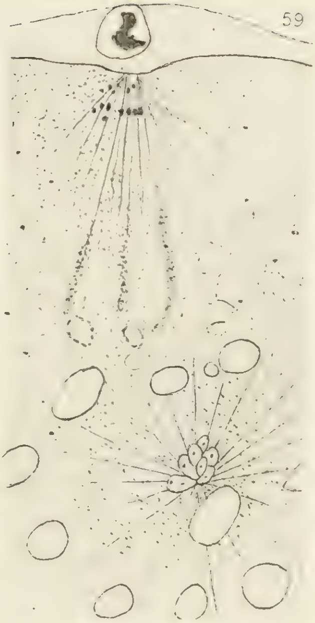

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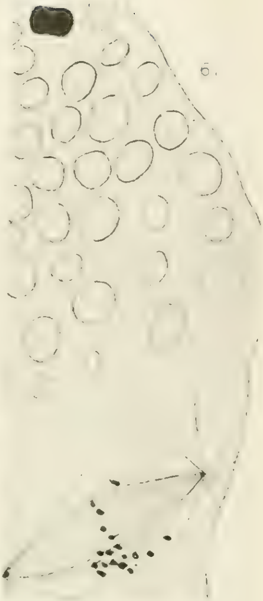

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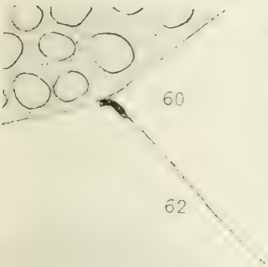

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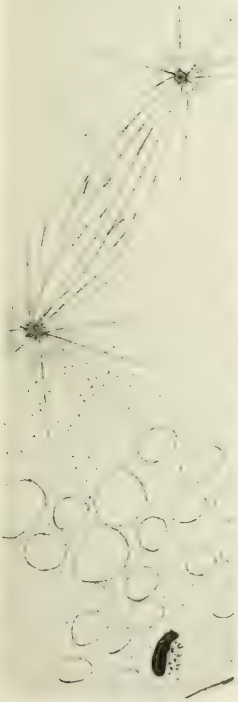

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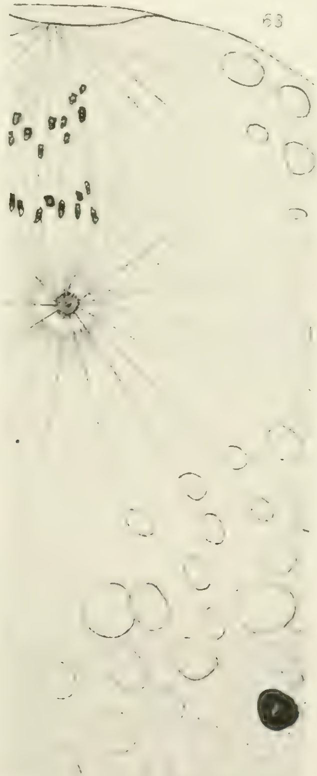

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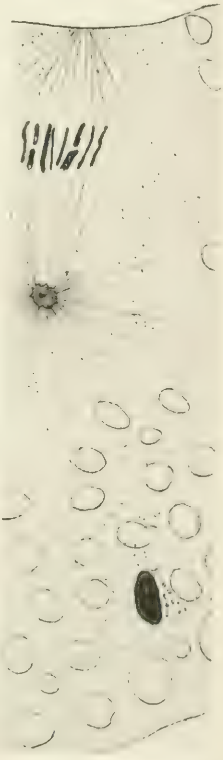


PLATE 10.

All figures photographic reproductions of sections magnified 670 diameters, and in reproduction reduced to about 450 diameters.

Figs. 65, 66. The central spindle passes through the germinative vesicle. The chromatin exists in the form of chromosome vesicles. Taken from the ovotestis. Iron hæmatoxylin and Bordeaux red. (Compare Plate 2, Fig. 9.)

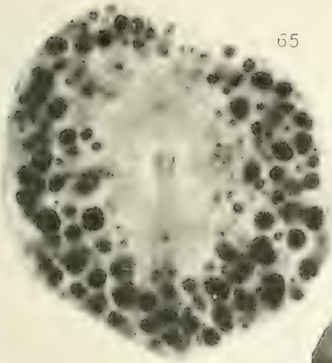
Fig. 67. Outline of germinative vesicle has disappeared. Chromosomes lie free in equatorial plane of spindle. Granules of cytoplasm arranged in rows about spindle poles. Taken from ovotestis. (Compare Plate 3, Fig. 13.)

Fig. 68. Metaphase of first maturation. The sphere-substance is differentiated into a light-colored medullary and a more deeply staining cortical layer. Mitotic figure moving peripherally. Iron hæmatoxylin and Bordeaux red. (Compare Plate 6, Fig. 26.)

Fig. 69. Anaphase of first maturation, shorter or direct process. Iron hæmatoxylin. (Compare Plate 6, Fig. 30.)

Figs. 70, 71. Unusually large first polar cell, *in mitosis*. Egg in the beginning anaphase. The focus in Figure 71 is such as to show the spindle in the polar cell, but not in the egg. (Compare Plate 6, Fig. 37.) Iron hæmatoxylin.

Fig. 72. Metaphase of first maturation. Two centrioles in deep centrosome. Chromosomes partly enclosed in a nuclear wall. Iron hæmatoxylin. (Compare Plate 7, Fig. 38.)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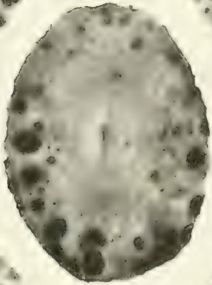
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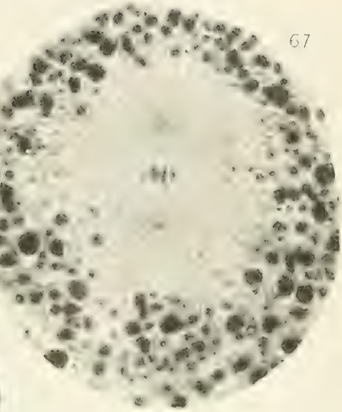
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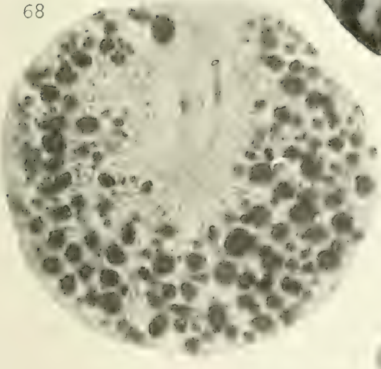
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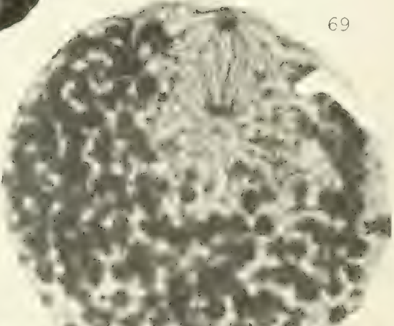
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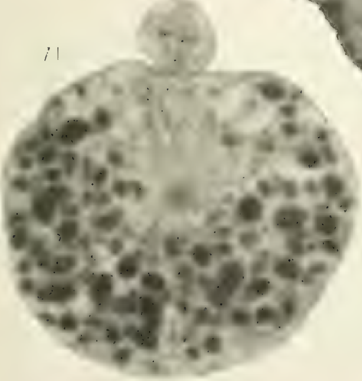
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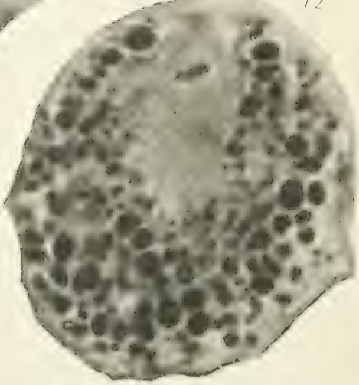
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PLATE 11.

All figures photographic reproductions of sections magnified 670 diameters, and in reproduction reduced to about 450 diameters.

Fig. 73. Prophase of second maturation figure; longer or indirect process. Chromosomes surrounded by a nuclear membrane. Achromatic figure surrounded by the medullary and cortical layers of the sphere. Iron hæmatoxylin. (Compare Plate 7, Fig. 42.)

Fig. 74. Prophase, second maturation, longer process. The achromatic figure is still enclosed in the wall of the parent centrosome. Chromosomes in two groups (not in focus), each with nuclear membrane. Iron hæmatoxylin and Bordeaux red. (Compare Plate 7, Fig. 41.)

Fig. 75. End view of second maturation spindle focused at the level of deep (polar-cell) end of the spindle. Same stage as that of Figure 73.

Fig. 76. The central spindle between the two asters has almost disappeared; it is represented by a wavy deeply staining band. Iron hæmatoxylin and Bordeaux red. (Compare Plate 7, Fig. 46.)

Fig. 77. The vesicular nucleus, containing the chromosomes, lies midway between the two asters, but the second maturation spindle has not been formed. Brazilin. (Compare Plate 7, Fig. 47.)

Fig. 78. Polar view of the deep centrosome of second maturation figure. Same stage as that of Figure 73. (Compare Plate 7, Fig. 38.)

Fig. 79. The nuclear membrane has disappeared, leaving the chromosomes free in the cytoplasm, the granules of which are arranged into rows. Fine fibres have grown out from the chromosomes a short distance toward the poles. Brazilin. (Compare Plate 7, Fig. 49.)

Fig. 80. Metaphase second maturation, longer or indirect process. Brazilin. (Compare Plate 8, Fig. 55.)

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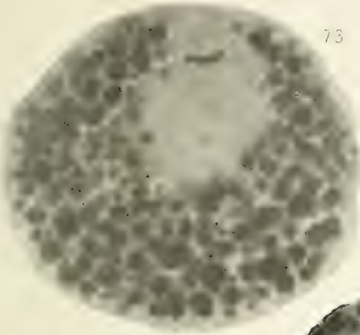

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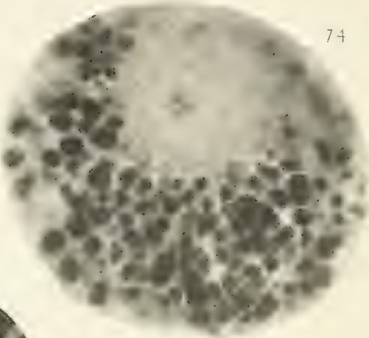

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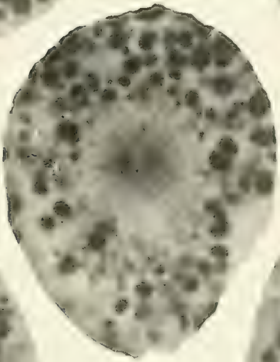

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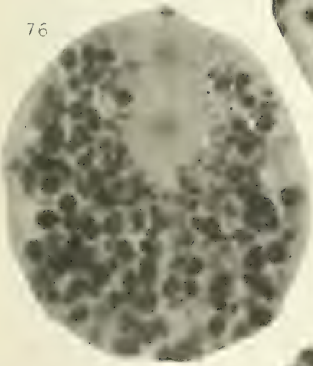

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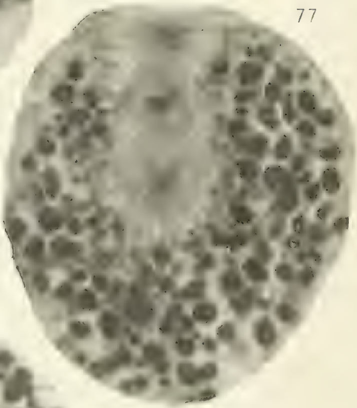

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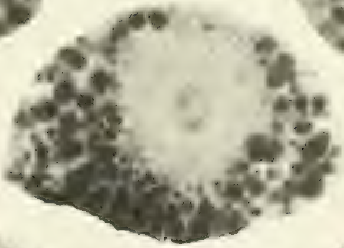

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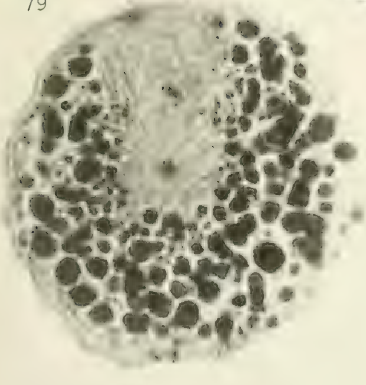

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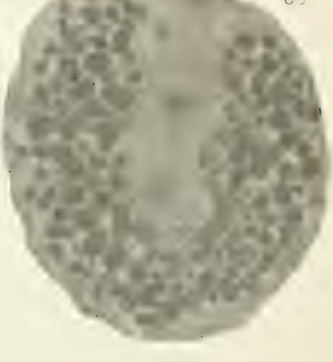


PLATE 12.

Fig. 81. First cleavage spindle. The chromosomes are becoming arranged in the equatorial plane of the spindle. The coarse chromatin granules are becoming diffused in the cytoplasm. × 1680.

Fig. 82. Transverse division of chromosomes in first cleavage. × 1224.

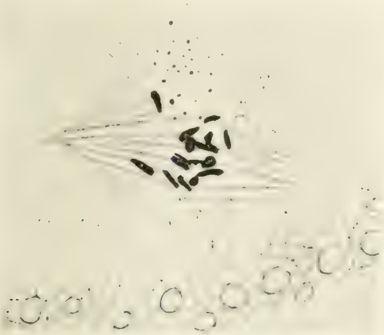
Fig. 83. Anaphase of second maturation division. The outer centrosome has disappeared, and the inner one is very faint (too prominent in the reproduced figure). Brazilin. × 1224.

Fig. 84. The chromosomes of the female pronucleus have become vesicular and lie in a cluster around the fading centrosome. The male pronucleus has a thick wall and contains chromatin granules and linin fibres. Iron hæmatoxylin and Bordeaux red. × 1224.

Fig. 85. Astral rays of the deep second maturation aster still persist. Male and female pronuclei lie close together; each much lobed owing to incomplete fusion of the nuclear vesicles, and containing a definite linin network. Iron hæmatoxylin. × 1224.

Fig. 86. First cleavage spindle. Compare description of Figure 81.

Figs. 87, 88. Prophase of first cleavage. The cleavage asters are not connected with each other, but each is associated with a single pronucleus. The chromatin of the chromosomes is solid. The rest of the chromatin exists in the form of rings. Iron hæmatoxylin and Bordeaux red. × 1680.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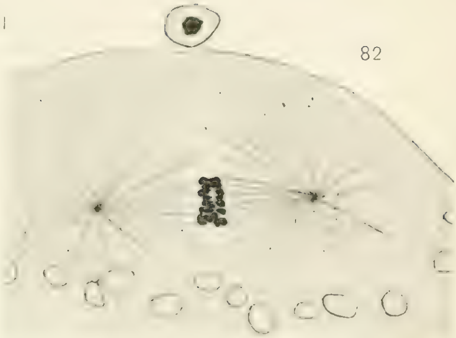
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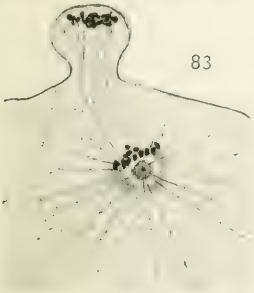
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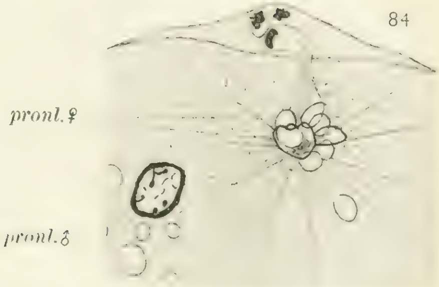
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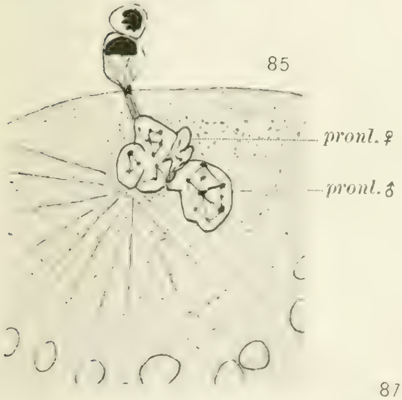
pronl.♂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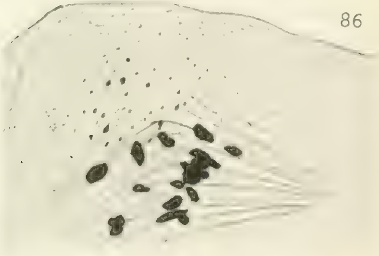
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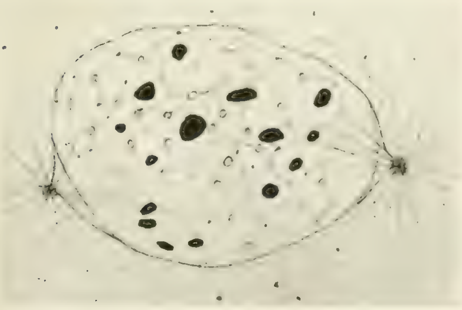
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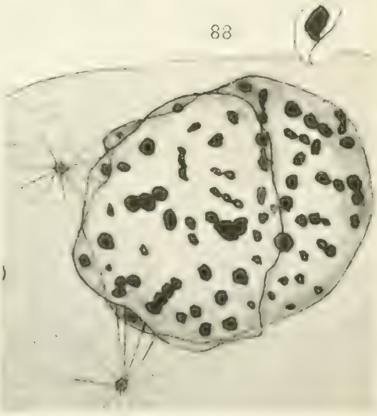
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PLATE 13.

All preparations stained with iron hæmatoxylin and Bordeaux red.

Figs. 89, 90. Prophase of first cleavage. The cleavage asters are not yet connected with each other, but are associated one with each pronucleus. The chromatin of the chromosomes is solid (Fig. 89) or vesicular (Fig. 90). The rest of the chromatin exists in the form of rings. There is a thin layer of sphere-substance around each centrosome. × 1680.

Fig. 91. Telophase of first cleavage. The chromosomes have fused into irregular nuclei. The astral rays directed toward the disappearing centrosome. × 1224.

Fig. 92. Anaphase of first cleavage. Part of the chromosomes vesicular. Centrosome still present, but irregular in outline. Thickenings on interzonal filaments. × 1224.

Fig. 93. Prophase of first cleavage. The solid black bodies are chromosomes. The cytoplasm in the region of the deutoplasm takes a deep stain. × 1680.

Fig. 94. Centrosome, cut parallel to axis of spindle, composed of centriole and centroplasm. Sphere-substance differentiated into medullary and cortical layers. × 1680.

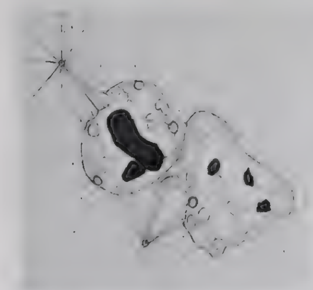
Fig. 95. Composite figure, from three sections of the incompletely fused nuclear vesicles derived from the chromosomes. First cleavage. × 2733.

Fig. 96. Prophase of second cleavage. Cleavage asters independent of each other, and lying at different levels in the section. × 1224.

Fig. 97. Quiescent nuclei in two-cell stage. Interzonal filaments and midbody present. × 1224.

Fig. 98. Prophase of third cleavage. Asters independent of each other. Cytoplasm takes a deep stain in region of deutoplasm. × 1224.

89



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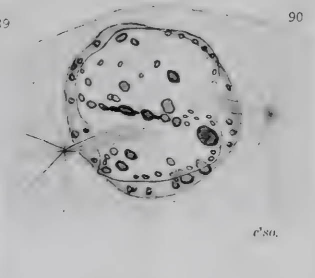

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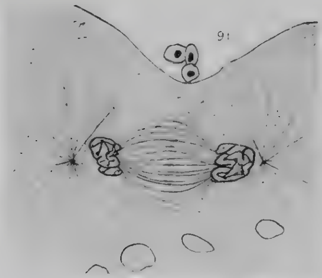

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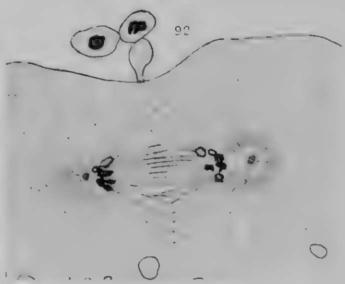

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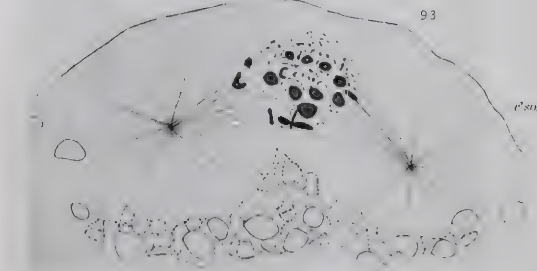

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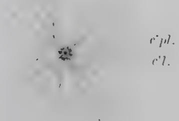
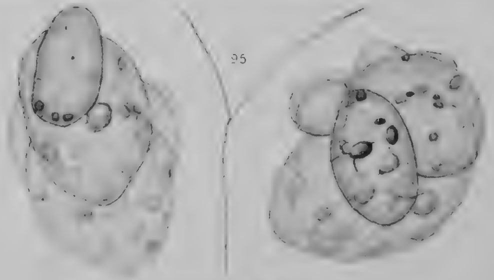

st.med.
c'pl.
c'l.

95



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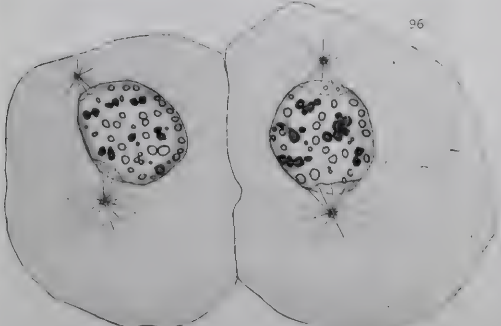

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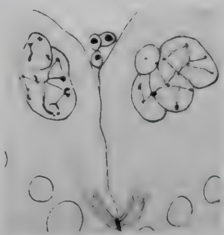

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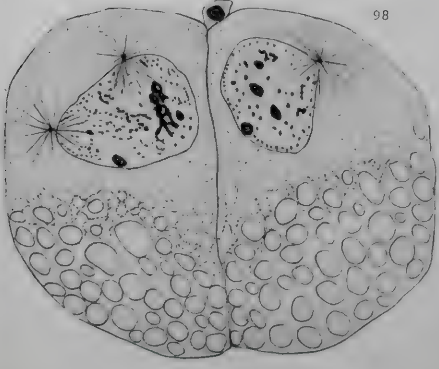

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